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TISSUE CULTURE

published mainly in such journals as the *Chinese Journal of Physiology*, the *Chinese Medical Journal, Journal of the Chinese Chemical Society, Nutrition Bulletin of Peking Agriculture Institute, Bulletin of the Institute of Biology of the Sino Academia*, etc. In 1926 nutrition workers participated in the organization of the Chinese Physiological Society, which was inaugurated in February of the same year; thus it was founded about 2 years prior to the inauguration of the American Institute of Nutrition. But it was not until December 1946 that nutritionists of the Chinese Physiological Society inaugurated the first Chinese Nutrition Society and subsequently published the *Chinese Journal of Nutrition*. Prior to that time, the *Chinese Journal of Physiology* published many articles on nutrition.

The years from 1938 to 1950 in the history of Chinese nutritional work might be termed the period of hard struggle, when in the first 7 years our country was invaded by the Japanese. Universities, colleges, and research institutes were moved to the interior and nutritional investigation was continued under very difficult conditions. In spite of the lack of adequate facilities such as laboratory space and equipment, nutritional work was carried on in temporary quarters. Prevalence of nutritional deficiency diseases due to the lack of proper foods prompted greater effort in nutritional studies. Two national nutrition conferences were called to discuss the basic nutritional problems of the country in 1941 and 1945, with the presentation of a number of nutritional research papers and the formal adoption of the first Chinese dietary nutrition requirements that were written by the Nutrition Committee of the Chinese Medical Association in 1938 (4).

After the surrender of the Japanese invaders in 1945, universities and research institutes were moved back to their original sites. Even though nutritional problems had become more acute because of the politically unsettled conditions and the difficult economic situation, urgently needed nutritional studies did not progress as they should have. The nutritional status of the great mass of people remained deplorable.

Soon after the overthrow of the old regime and the establishment of the People's Republic of China, great attention was paid by the government to the nutritional problems of the people. The nutritional status of the people improved considerably. Nutritional deficiency diseases became rare in cities, though in some remote rural areas, owing to ignorance and improper intake of foods, some deficiency diseases such as pellagra, infantile beriberi, goiter and xerophthalmia were still present. Studies in basic and applied nutrition were encouraged and promoted by the new government, and much was accomplished. In April of 1954, the Chinese Nutrition Society merged with the Physiological, Biochemical, Pharmacological, and Biophysical Societies to form the Chinese Society of Physiological Sciences and held local monthly as well as national annual scientific meetings. In 1956 the reorganized *Chinese Journal of Nutrition* appeared as a quarterly. Theoretical, basic, and applied nutrition progressed rapidly until the cultural revolution was usurped by Lin Biao and the "Gang of Four," and nutritional work was much deprecated. Many nutritional workers were forced to suspend activities or to undertake other jobs. We actually lost over 10 years of otherwise very productive work. Nutritional Society activities, including the journal publication, were forced to suspend operations. After the downfall of the "Gang of Four," nutritional work was gradually revived, and research resumed. In October of 1978, the Chinese Society of Physiological Sciences held a one-week conference, in which papers on "recent advances in nutrition," on "diet and nutrition during the 'New Grand March,' " and on "nutritional education and research" were presented and discussed. Over 70 research papers were submitted to the nutritional section of the conference. In September of 1979 a national nutrition conference was held in Cheng Du in which important problems of nutrition, including dietary nutritional requirements and allowances, new dietary protein and lipid sources and nutrition for children and the aged were discussed. In

addition, there were over 180 research papers on subjects relating to basic nutrition, childhood nutrition, nutrition and diseases, new sources of foods, the effect of processing and preservation on food values, nutritional survey and food hygiene submitted to the conference. In October of 1979 a national conference on nutritional requirements and dietary allowances was convened in Tian Jin, and a tentative, revised list of nutritional allowances was formulated and discussed. The final form of the revised recommended dietary allowances was approved at the Third National Nutritional Scientific Conference held in May 1981, and topics of special interest were also discussed, research papers (over 300 were submitted) were presented, and the National Nutrition Society was formally inaugurated. Thus nutritional work has been reinvigorated, and it is hoped that before long nutrition activities will be expanded greatly to meet the actual need of the whole country.

Past Nutrition Studies. The first publication on nutrition investigation in China was "Dietary Studies in Shantung" by Adolph in 1913 (5). This was followed by a paper on the analysis of litchi by Read (6) in 1918 and a paper on a study of the different kinds of milk in Kwangtung in the same year (7). Papers on the nutritive values of soybean products and analyses of Chinese foods appeared in 1920 (8) and 1921 (9). During subsequent years, many more papers on diet and nutrition in China were published on varied subjects: (i) dietary studies in different parts of China, in urban and rural areas, among different groups of people (students, institution workers, factory workers, farmers, soldiers and laborers) were undertaken; (ii) analyses on protein, fat, carbohydrate, mineral, vitamin, water, and crude fiber content of foods in different parts of China were done. Considerable variation in composition of foods from different localities, especially vitamins and certain minerals, were found. Owing to the vastness of our country, great differences in climatic conditions, soil composition, use of fertilizers, selection of genetic strains, agricultural methods, etc., such variations would be expected. Other topics of research were: (iii) digestion, utilization, and metabolism among different groups of nhabitants and in healthy and diseased individuals; (iv) protein and amino acids in nutrition; (v) vitamins in foods and nutrition; (vi) minerals in foods and nutrition; (vii) nutrition in vulnerable population groups, particularly children; (viii) nutritional value of vegetarian diets; (ix) dietary requirements and allowances for the Chinese; (x) nutritional deficiency diseases among Chinese under different circumstances such as peacetime, wartime, famine years, political and economic disturbances, particularly deficiencies in vitamins A, D, B_1, C, and nicotinic acid as well as protein and iodine deficiencies. Patients with deficiencies were found to be much more numerous during difficult times than during peaceful times. Introduction of iodized salt or other iodine-containing foods greatly reduced the incidence of goiter in endemic areas. In addition to the finding of iodine-deficiency goiter, goiterogenic substances were found in certain foods (10).

Aside from the above nutritional studies there were also organizations or units for the promotion of applied nutrition, nutritional propaganda, and education, with provision for free distribution of food at times of emergency and supplementary feeding of the poor to combat deficiency diseases (11, 12).

Following the establishment of the People's Republic of China in 1949, considerable work on basic nutritional research, applied nutrition, and dietary therapy was conducted at various institutions. The achievements or results of studies were summarized in several papers published in the 10th anniversary publication of the Ministry of Health (13) and various science journals such as the *Progress in Physiological Sciences* (14), the *Chinese Medical Journal,* and the *Journal of the Chinese Chemical Society*. In brief, advancement was attained in investigations on dietary and nutrition surveys, on dietary and nutrition requirements and allowances, that formed the basis for the first revision of Chinese di-

etary allowances; nutritional deficiency diseases, their treatment and prevention; dietary therapy and special diets for patients with various diseases; food composition analyses including amino acids, fatty acids, vitamins, and toxic substances; improvement of nutritional survey techniques; influence of cooking, storage, processing, and preservatives on the nutritive values of foods; effects of environment on nutrition, etc. It was found, for instance, that certain algae were suitable as a supplementary food (15), that low doses of gamma ray radiation for preservation of foods (grains) did not influence the nutritive values nor impart any toxicity to the irradiated foods (16), and that the nicotinic acid in corn was found to exist in bound form and could be liberated as the free form by the simple addition of sodium bicarbonate during cooking and thus become effective in preventing pellagra (17). Keshan disease, an endemic chronic myocardiopathy was found to be related mainly to a lack of sufficient selenium (18). The soil and foods of the endemic areas as well as the blood and hair of the inhabitants were found to be very low in selenium content, and oral intake of small amount of selenium salt greatly lowered the incidence of Keshan disease (19).

Current Nutritional Studies. Both basic (theoretical) and applied nutritional studies are being carried out at institutions throughout the country on such general topics as:

1. The relation between diet, nutrition, and diseases such as studies on foods lowering blood lipids and their relation to coronary heart diseases; on the use of amino acid mixtures or fish protein concentrate in patients with injuries and in malnutrition cases; further studies on the use of the hybrid corn from opaque-2 with a Chinese variety for the prevention and treatment of pellagra; on the cause of "hyperiodine goiter," etc.
2. Food fortification with stable vitamins, amino acids, calcium, and iron in health and disease.
3. Food hygiene, food contamination, food toxicology, insecticide residues in relation to nutrition.
4. Further studies on food compositions in newer foods, especially with regard to amino acids, fatty acids, vitamins, and trace elements.
5. The interrelationship between nutrients in diets of different compositions in different ethnic groups throughout the country.
6. Nutritional requirements and dietary allowances of various age and sex groups among different ethnic groups.
7. Nutritive values of newer vegetable proteins and novel foods.
8. The relation of diet and nutrition to cancers.
9. Further studies on nutritional factors in Keshan disease.
10. Nutritional problems in relation to agriculture and animal husbandry.

In addition to the above topics, there are certain problems that deserve further consideration:

1. The art of Chinese cooking is well known throughout the world as being tasty, colorful, and artistic, but the influence of the mode of processing and cooking on nutritive values has not yet been well studied.

There are many old methods of preserving foods at times of abundance to meet the need at times of want. Preservation alters the taste and appearance of foods. So far changes in nutritive values during preservation have not been thoroughly investigated.

It will be very interesting and of practical importance to make a thorough study of the various methods of preparing and preserving foods in China with regard to any possible alteration in nutritive value.

2. The incidence of certain cancers is particularly high in certain parts of China. There is every indication that it is related to food habits, the prolonged intake of harmful ingredients in certain foods or the continual lack of certain protective nutritive substances in diets. Further studies may yield fruitful results.
3. Recent surveys indicate that goiter and cretinism are still present in certain

parts of China. This is due mainly to a lack of proper enforcement of the use of iodized salt in endemic areas; it had been the practice in certain districts to roast salt before use, thus causing loss of the added iodine.

It has been noted recently that in certain oil field areas, deep well water containing a very high level of iodine can also induce goiter. The cause of this needs further study. The possibility of the presence of other goiterogenic agents has yet to be ruled out.

4. The lack of selenium has been implicated in Keshan disease, but the presence of other factors, nutritional or otherwise, might play a part. Progress has been made in the elucidation of the multiple factors in its etiology but further clarification is still needed.

5. The last country-wide nutritional survey was made over 20 years ago. The results of that survey were not well assessed. The present nutritional status of the whole country is yet unknown. A country-wide survey in 1982 is planned, the goal of which will be to obtain a concrete idea of the actual nutritional status to be used as a basis for the planning of food production and distribution.

6. Owing to the differences in climatic conditions, soil qualities, fertilizers used, and methods of cultivation, the nutrient composition of foods of different localities varies considerably. Thus the present food composition tables cannot be used in different districts throughout the country. Many more food-analysis data are required, especially with regard to trace elements, amino acids, fatty acids, and vitamins.

7. There are differences in the dietary habits and ways of livelihood of different tribes of people in different localities of the country, and the dietary requirements likely vary. Many more studies are thus needed in order to establish a better understanding of the real nutritional need of every individual and the population as a whole.

8. The production of food in our country at present does not keep up with the growth of the population (birth control is already in practice). It is necessary therefore to search for newer foods (especially protein-rich food sources), to conserve food (avoid waste), and to use the right combination of foods in order to obtain the highest degree of digestion and utilization so that no food is wasted. Obesity due to overeating and certain associated diseases are not uncommon now. Hence much more nutritional education is needed, both in the training of nutritional workers and in the dissemination of nutritional knowledge to the masses. There is also a need for closer cooperation between nutritional workers and food producers (agriculturists, food manufacturers, and animal husbandry workers).

1. Huang Di Nei Jin Su Wen, revised by Kao Bao Heng *et al.* People's Health Publishing House, Vol 7(8):p149, 1963.
2. Hou HC. Chinese ancient books on food and nutrition. Nat Med J China 22(11):1015–1026, 1936.
3. Hou HC. Dietary principles in ancient Chinese medicine. Chin Med J 57:347–352, 1938; Beriberi in ancient Chinese medical literature, Chin Med J 58:302–313, 1940.
4. Committee on Nutrition. Chinese Medical Association Special Report, Ser. No. 10. Shanghai, 1938.
5. Adolph WH. Diet studies in Shangtung. China Med J 27:1013, 1913.
6. Read BE. Analysis of Litchi. J Amer Chem Soc 40:817, 1918.
7. Levine CO, *et al.* A study of the different kinds of milk production in Kwangtung. China Med J 32:536, 1918.
8. Wilson SD. A study of Chinese foods. China Med J 34:503, 1920.
9. Embrey A, Wang TC. Analysis of some Chinese foods. China Med J 35:247, 1921.
10. Hou HC. Thyroid enlargement following liver feeding in rats. Proc Soc Exp Biol Med 43:753, 1940.
11. Hou HC. Chinese dietary standard and some dietary problems among war refugees. C R Congr FEATM, 10th, Hanoi, November 26–December 2, 1938.
12. Hou HC. Nutrition supplements for refugee children. Chin Health Mag 2(4), 1940.
13. Collected Papers of Medical Researches in Celebrating the 10th Anniversary of the Founding of New China. Peking, Ministry of Health of the People's Republic of China, Vol 1, December 1959.

14. Hou HC. Ten years achievement in vitamin research. Prog Physiol Sci 3(2):105–115, 1959.
15. Hou HC, *et al.* Studies on the nutritional values of Chinese algae. Chin Health J 8(1): 29–34, 1963.
16. Hou HC, *et al.* On the question of toxicity of gamma ray irradiation upon grains. Technique in nuclear energy—Applied radiation with isotope. Pp167–173, 1960.
17. Wang GY *et al.* Studies on improving the utilization of nicotinic acid in corn. Chin J Physiol 29:197, 1966.
18. Keshan Disease Research Group, Chinese Academy of Medical Sciences. Studies on the epidemiological characteristics on the etiological relationship of selenium to Keshan disease. Nat Med J China 59(8):451, 1981.
19. Research Laboratory of Keshan Disease Group, Xian Medical College, *et al.* Observation on the effects of sodium selenite for preventing acute Keshan disease. Nat Med J China 59(8):457, 1979.
20. Zhu XY. Development of ongoing research work on endemic goiter and cretinism. Nat Med J China 60(12):705–707, 1980.

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Cold Agglutinin Antibody in a Hyperimmune Erythrocyte Antiserum (41469)

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Abstract. To examine whether an innocuous, nonbacterial-associated immunogen could generate a cold agglutinin autoreactive antibody response, rabbits were hyperimmunized with sheep erythrocytes. Of five animals immunized, one developed an autoreactive antibody population which agglutinated autologous and allogenic erythrocytes at temperatures below 37°. The cold agglutinin antibody activity was present in the IgM class immunoglobulin fraction since only the excluded volume of a Sephacryl S-200 serum fractionation, and purified IgM, contained detectable activity. The cold agglutinin antibody was hemolytic in the presence of guinea pig complement, since whole serum, and purified IgM, sensitized rabbit erythrocytes for lysis in a biphasic temperature hemolysis assay. In hemolytic inhibition assays, the cold agglutinin antibody was sugar specific. The relative sugar ligand specificity in this assay was shown to be *N*-acetylgalactosamine > melibiose > galactose > lactose. The hyperimmune anti-sheep erythrocyte serum agglutinated both sheep erythrocytes and Group C streptococcal vaccine at 4° and at 37°. These data suggest certain animals can respond to nonbacterial-associated immunogen with antibody specific for the immunogen, but which may cross-react with autologous sugar determinants.

Cold agglutinin (autoreactive erythrocyte) antibodies have been shown to be associated with infectious illness and other human disease (1–5), and may appear spontaneously in certain strains of mice (6). Also, cold agglutinin antibodies may appear during hyperimmunization of rabbits with certain bacterial vaccines (7–9). Because of these associations, it has been of interest to determine whether these particular autoantibodies arise in response to the infectious or immunizing agent or whether they arise nonspecifically as one result of general immune stimulation.

A recent investigation by our laboratory revealed a cold agglutinin antibody response in rabbits hyperimmunized with Group C streptococcal vaccine (10). The Group C streptococcal cell wall carbohydrate structure carries determinants identical to those present on the glycan portion of the Forssman glycolipid of sheep erythrocytes. These determinants are α-anomerically linked *N*-acetylgalactosamine disaccharide residues. These same residues are the immunodominant structures present on Group C carbohydrate (11). Both IgM and IgG cold agglutinin antibodies isolated from Group C streptococcal antisera reacted with *N*-acetylgalactosamine and Group C carbohydrate. These data therefore suggested that these particular cold agglutinin antibodies were induced in response to the Group C streptococcal cell wall carbohydrate.

The present investigation was initiated to examine whether a bacterial vaccine was necessary to induce the cold agglutinin response, or whether a similar antibody response might be generated by hyperimmunization of rabbits with sheep erythrocytes.

Materials and Methods. *Vaccine preparation and immunization protocol.* Preparation of vaccine was according to Lancefield (12). Briefly, bacteria were cultured overnight in Todd–Hewitt broth and vaccine prepared from washed, pepsin-digested cells. The procedure used for immunization was according to Herd and Spragg (13). Rabbits were immunized three

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times per week for 4–5 weeks, rested 3 months, and again immunized.

Sheep erythrocyte immunization protocol. Sheep erythrocytes in modified Alsever's solution were washed three times in 0.15 *M* NaCl and pelleted by centrifugation at 800 g. Red blood cells were resuspended in 0.15 *M* NaCl to 10% (v/v) and 1.0 ml injected iv three times per week for 5 weeks into five animals. Rabbits were bled from the central ear artery each week.

Hemagglutination assay. Allogenic rabbit erythrocytes were collected in Alsever's solution and washed in 0.02 *M* phosphate, 0.15 *M* NaCl buffer, pH 7.2, made 1% (w/v) in bovine serum albumin. Cells were resuspended in this buffer to 0.5% (v/v) and distributed in "v" type microtiter plate wells. The wells contained an equal volume of twofold-diluted whole or Sephacryl S-200 fractionated sheep erythrocyte antisera. Microtiter trays were stored at 4° overnight and the reciprocal of the highest dilution which yielded positive agglutination was recorded as the cold agglutinin antibody titer.

Biphasic temperature hemolytic assay. This assay has previously been described in detail (10). Essentially, a pretitrated excess amount of guinea pig complement was added to a 0.25% (v/v) suspension of rabbit erythrocytes in the presence of twofold serially diluted whole or fractionated antisera. After incubation at 4° for 60 min and 37° for 60 min the amount of hemolysis was determined by supernatant volume absorbance measurements at 413 nm. Inhibition in this assay was performed using a pretitrated amount of cold agglutinin antibody sufficient to yield 50% hemolysis under conditions described above. Preincubation of increasing amounts of varying inhibitors with this particular amount of antibody was performed for 3 hr at 4° prior to assay. Inhibition was calculated relative to an uninhibited control.

Polysaccharide preparations. Group C streptococcal carbohydrate was purified according to an acidified sodium nitrite procedure provided by E. Gotchlisch, The Rockefeller University, New York (personal communication). Type III pneumococcal polysaccharide was purified according to Campbell and Pappenheimer (14). Type XIV pneumococcal polysaccharide was the generous gift of Eli Lilly and Company, Indianapolis, Indiana.

Results. Rabbit erythrocyte agglutinating activity at 4° or 37° was absent from preinoculation sera, while at these temperatures the same sera agglutinated sheep erythrocytes to a 1:4 dilution. After immunization, all rabbits produced sheep red blood cell-reactive antibodies (reactive at both 37° and 4°). Only one rabbit, however, produced antibodies reactive against rabbit erythrocytes; and, reactivity was detectable only at 4°. For this particular rabbit's antiserum, the sheep erythrocyte hemagglutination titer increased from 1:4 to 1:1024 during the fifth week of immunization. In contrast, hemagglutination reactivity of whole serum with rabbit erythrocytes occurred during the first, second, and third weeks only, and was relatively weak (highest titer < 1:32), and occurred only at 4°. Reactivity with both human adult O-positive and cord blood erythrocytes occurred in the warm (titer < 1:4) and increased in the cold (titer < 1:8). None of the remaining animals' sera reacted with human or rabbit erythrocytes when tested at either 37° or 4°. Hemolytic activity toward rabbit erythrocytes in the biphasic temperature hemolytic assay reached, however, a titer of 1:256 during the third week of immunization. Since most cold agglutinin antibodies are of the IgM class, the anti-sheep red cell antiserum was fractionated into 19 S (excluded) and 7 S (included) components by Sephacryl S-200 chromatography and examined for erythrocyte autoreactivity.

Figure 1 shows the results of hemolytic assays performed upon individual Sephacryl S-200 fractions. As shown, it is clear that all of the cold agglutinin activity was confined to the excluded column fraction; these results in addition to Ouchterlony analysis with goat anti-rabbit μ chain antiserum and polyvalent goat anti-rabbit Ig antisera, showed that IgM was the only immunoglobulin component in these fractions. That the cold agglutinin activity was

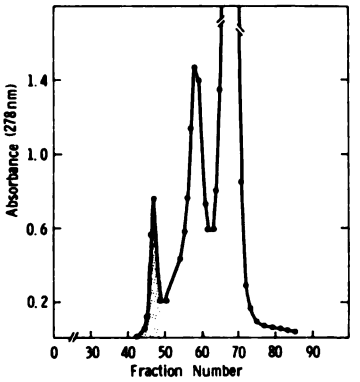

FIG. 1. Fractionation of 4.0 ml rabbit sheep erythrocyte antiserum on a 2.8 × 100-cm Sephacryl S-200 column. Shaded area represents fractions which exhibited cold agglutinin activity. Fraction volumes were 4.1 ml.

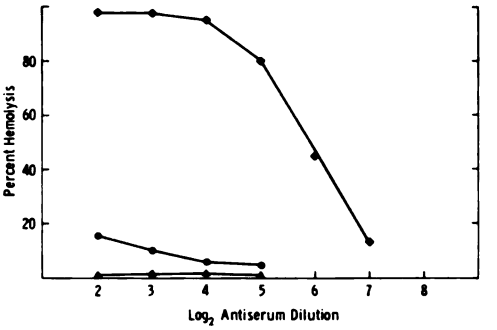

FIG. 2. Cold agglutinin activity in biphasic temperature hemolytic assay, using whole (■), Group C Streptococcal vaccine absorbed (●), or sheep erythrocyte-absorbed (▲) sheep erythrocyte antiserum.

associated with sheep erythrocyte determinants was shown by absorption experiments. Whole antiserum was absorbed once in the presence of one-half the serum volume of packed erythrocytes. In addition, the same procedure was performed using Group C streptococcal vaccine. After absorption, each solution was titrated in the biphasic temperature hemolysis assay. Shown in Fig. 2 are the results of these assays. As depicted, both absorption procedures were effective in depleting the antisera of hemolytic activity toward rabbit red blood cells. Absorption with Type III pneumococcal vaccine did not alter the original activity. Because of the apparent reactivity of the cold agglutinin material with sheep erythrocytes and Group C streptococcal vaccine, further examination involved fractionated material in ligand inhibition experiments. Cold agglutinin antibody, after fractionation by Sephacryl S-200 chromatography, was concentrated and the IgM antibody titrated to yield 50% hemolysis in the biphasic temperature hemolysis assay. This amount of antibody was subsequently preincubated in the presence of various saccharide compounds as inhibitors. Shown in Table 1 are the results of these assays. Clearly, of the polysaccharides tested, Group C streptococcal carbohydrate was the best inhibitor of hemolysis. More than 90% inhibition was effected in the presence of 100 ng carbohydrate. At a similar concentration Type III pneumococcal polysaccharide was not inhibitory. Of the individual sugars tested, *N*-acetylgalactosamine clearly yielded the most inhibition. As seen by the data in Table 1, approximately 200 μM *N*-acetylgalactosamine was sufficient to cause 50% inhibition of lysis while greater than 1200 μM melibiose was required for equivalent inhibition activity. When lactose was tested, greater than 10 m*M* did not cause detectable inhibition of cold agglutinin antibody: erythrocyte interaction. Thus, lactose was the least effective inhibitor. Interestingly, galactose, although significantly less reactive than *N*-acetylgalactosamine, was a more effective inhibitor than lactose (approximately 7 m*M* required for 50% inhibition).

Discussion. The demonstration that antibody reacts with a specific immunogen, implies antibody has arisen in direct response to the immunogen, and not indirectly as a result of nonspecific B lymphocyte activation. It is possible, however, that under hyperimmune conditions, non-antigen-specific soluble activation factors may stimulate nearby lymphocytes to elicit a primary response. Certain cold agglutinin antibody responses may fall into this category. For example, cold agglutinin antibodies which appear during infectious illness, or during experimental animal hyperimmunization can be shown to react with

TABLE I. INHIBITION CHARACTERISTICS OF VARIOUS SACCHARIDES AND POLYSACCHARIDES IN BIPHASIC TEMPERATURE HEMOLYTIC INHIBITION ASSAY

Inhibitor	Amount required for 50% inhibition	
	mM	mg
N-Acetylgalactosamine	0.22	—
Melibiose	1.78	—
Galactose	7.08	—
Lactose	No inhibition at 10.0	
Group C carbohydrate[a]	—	42
Type XIV carbohydrate[b]	—	119

[a,b] These materials were of heterogeneous molecular weight; therefore, weight values are provided.

both bacterially associated and autologous erythrocyte-associated structures. Partially because of the antigenic complexity of these systems, it has been particularly difficult to determine whether (1) non-antigen-specific immune stimuli, (2) bacteria or products in association with erythrocytes, or (3) erythrocyte cross-reactive bacterial determinants, have led to production of cold agglutinin antibodies. The present observation that sheep erythrocytes can, albeit rarely, lead to a cold agglutinin antibody response, suggests a cross-reactive antibody may be generated.

It is important to note that results of the present work which utilized an innocuous agent, sheep erythrocytes, as the immunogen are similar to those obtained in other investigations which utilized bacterial vaccines. As was observed in the present study, galactose-containing ligands appeared to react best with the cold agglutinin antibody. It is also important to note, however, that the cold agglutinin antibody described in the present investigation is clearly different from the antibody described earlier in association with Group C streptococcal vaccine immunization (10). First, a significantly different sensitivity to lactose inhibition is evident since lactose was not inhibitory in the present case at a concentration which inhibited 50% of the hemolytic activity of the previously described and characterized IgM antibody. In addition, these cold agglutinin antibodies did not discriminate between I or i determinants. Human adult and cord blood O-positive erythrocytes were equivalently agglutinated at 4°. Further, agglutination of these cells occurred at 37°. Since sheep erythrocytes do not carry detectable I or i determinants, these data suggest that reactivity with human and rabbit erythrocytes by these cold agglutinin antibodies does not involve specific discriminatory recognition of I or i determinants. This observation does not, however, exclude the possibility that these sugar-reactive cold agglutinin antibodies react with galactosyl moieties on the I or i oligosaccharide complex. Similar to the cold agglutinin-containing antistreptococcal sera reported in a previous investigation, the single antiserum in the present study reacted in both the warm and cold with sheep erythrocytes, but only in the cold with rabbit erythrocytes. The Forssman glycolipid glycan portion is identical to the immunodominant Group C streptococcal carbohydrate determinants. It appears, therefore, that an immune response to Forssman antigen determinants could explain the source of rabbit erythrocyte autoreactivity. Whether other antigens were involved in the present response is not known.

The cold agglutinin antibodies presently described reacted best, but not exclusively, with *N*-acetylated-α-anomers. This interpretation is based upon the data which clearly revealed preferential reactivity with *N*-acetylgalactosamine versus galactose and melibiose versus galactose and lactose. Apparently, the 100% β-anomeric structure present in the β 1→4 linkage of the lactose molecule prevented significant reactivity with the antibody combining site. Further,

the fact that lactose is a disaccharide per se is not relevant to the absence of inhibition observed in the presence of this component, since melibiose was significantly inhibitory.

That cold agglutinin antibody may appear during immunization with erythrocytes in addition to immunization with various bacterial vaccines, strongly suggests the response is specific for sheep erythrocytes and is independent of bacterial components in association with autologous erythrocyte membranes. These data therefore suggest that a clearer understanding of erythrocyte autoreactive responses could be attained through careful use of defined carbohydrate immunogenic stimulation.

1. Pruzanski W, Shumak KH. Biologic activity of cold reacting autoantibodies. N Engl J Med 297:538–542, 1977.
2. Frank MM, Atkinson JP, Gadek J. Cold agglutinins and cold agglutinin disease. Annu Rev Med 28:291–298, 1977.
3. Schubothe H. Current problems of chronic cold hemagglutinin disease. Ann NY Acad Sci 124:484–490, 1965.
4. Harboe M. Cold Auto-agglutinins. Vox Sang 20:289–305, 1971.
5. Roelcke D. Cold agglutination, antibodies and antigens. Clin Immunol Immunopathol 2:266–280, 1974.
6. DeHeer DH, Linder EJ, Edgington, TS. Delineation of spontaneous erythrocyte autoantibody responses of NZB and other strains of mice. J Immunol 120:825–830, 1978.
7. Costea N, Yakulis VJ, Heller P. Experimental production of cold agglutinins in rabbits. Blood 26:323–340, 1965.
8. Costea, N, Yakulis VJ, Heller P. The mechanism of induction of cold agglutinins by *Mycoplasma pneumoniae*. J Immunol 106:598–604, 1971.
9. Lind K. Production of cold agglutinins in rabbits induced by *Mycoplasma pneumoniae, Listeria monocytogenes*, or streptococcus MG. Acta Pathol Microbiol Scand Sec B 81:487–496, 1973.
10. Colling RG, Brown JC. The appearance of IgM and IgG cold agglutinins in rabbits hyperimmunized with group C streptococcal vaccine. J Immunol 122:202–208, 1979.
11. Colligan, JE, Fraser B, Kindt T. A disaccharide hapten from streptococcal group C carbohydrate that cross-reacts with the Forssman glycolipid. J Immunol 118:6–11, 1977.
12. Krause RM. The search for antibodies with molecular uniformity. Advan Immunol 12:1–56, 1970.
13. Herd ZL, Spragg J. The occurrence of homogeneous antibodies and selection of κ light chains to streptococcal carbohydrates in Monash rabbits. Aust J Exp Biol Med Sci 50:225–243, 1972.
14. Campbell JH, Pappenheimer AM Jr. Quantitative studies of antipneumococcal polysaccharide antibodies Types III and VIII–I. Immunochemistry 3:195–212, 1966.

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Effects of Culture Age on PRL and GH Responses to Bromocriptine and Somatostatin from Primary Cultures of Rat Anterior Pituitary Cells (41470)

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Abstract. To study the effects of time in culture on prolactin (PRL) and growth hormone (GH) responses to exogenous stimuli, bromocriptine (10^{-7} *M*) or somatostatin (10^{-7} *M*) were added to primary cultures of dispersed rat anterior pituitary cells (DC). Cells which had been in culture for 3, 6, or 9 days were then incubated for 6 hr and the media were radioimmunoassayed for PRL and GH. The inhibitory effects of bromocriptine on PRL release (76% decrease) and somatostatin on GH release (62% decrease) from cultured cells were maximal on Day 3 and decreased with age of the culture. In addition, the inhibitory effect of bromocriptine on GH release (32% decrease) was slight but maximal on Day 3 and decreased with time in culture. In contrast, somatostatin showed slight and stable inhibition of PRL release (26 to 29% decrease) at each of the three time periods. At the end of each incubation, the media were replaced with Ham's F-10 medium lacking bromocriptine or somatostatin and the cultures were incubated again for 6 hr. Even after removal of these agents, the inhibitory effects of bromocriptine on PRL or somatostatin on GH release persisted at almost the same or a higher degree compared to the controls. On the other hand, the inhibitory effects of bromocriptine on GH or somatostatin on PRL disappeared almost completely after removal of the agents. The release of both PRL and GH into the media was greater in all cases, including the controls, during the second incubation. This may have been due in part to the stimulation caused by changing the media. The results suggest: (1) rat pituitary mammotrophs and somatotrophs may both possess receptors for bromocriptine and somatostatin (2) in the absence of hypothalamic control (i.e., *in vitro* system), these cells are most sensitive to both agents on Day 3, and (3) the rebound increases seen *in vivo* following these agents are likely caused by hypothalamic modulations.

The effect of the age of primary cultures on the course of basal hormone secretion and on the reponsiveness of anterior pituitary cells to exogenous stimuli has not been well studied. In addition, the mechanism of rebound increases in prolactin (PRL) and growth hormone (GH) secretion in rats and humans after cessation of treatment with inhibitory agents is not fully understood (1–7). To investigate these important questions, we used primary cultures of rat anterior pituitary cells and examined the basal secretion and the responsiveness of the mammotrophs and somatotrophs to bromocriptine and somatostatin on Days 3, 6, and 9 in culture.

Materials and Methods. *Dispersed cell cultures.* Male Sprague–Dawley rats (Charles River Corp., Wilmington, Mass.), weighing 200–250 g, were housed in an artificially illuminated (14 hr light, 10 hr dark) animal room for periods of 1 to 2 weeks before sacrifice. Food and water were available *ad libitum*. Twenty-three animals were killed at 09:00 hr by decapitation. The pituitary glands were removed aseptically under a laminar flow hood, the posterior lobes were discarded, and the remaining tissue was placed into a sterile petri dish (5-cm diameter) and minced to the size of 1 mm^3 in Hank's balanced salt solution without Ca^{2+} or Mg^{2+}. The minced pituitary tissue was washed gently with Hank's solution five times to remove blood cells. Then 5 ml of 0.1% trypsin (Grand Island Biological Co., GIBCO, Grand Island, N.Y.), solubilized in Hank's solution containing 0.1%

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bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.) was added to the petri dish, and the tissue was then transferred to a 35-ml Erlenmeyer flask. The solution was agitated gently by a stirrer for 10 min, and the supernatant, which contained dispersed pituitary cells (DC), was collected by filtration through lens paper (Matherson Scientific, New York) in an ice-cooled 50-ml Erlenmeyer flask. Then, 5 ml of Ham's F-10 medium supplemented with 10% fetal calf serum and 2.5% horse serum was added to the flask to block the action of the trypsin. The trypsinization and cell collection procedures were repeated five times. Finally, the cell suspensions were centrifuged for 5 min at 1000 rpm, and the cells were resuspended in Ham's F-10 medium. These cells were cultured in T-25 flasks (Corning, No. 25100) at 37° for 3, 6, or 9 days in an atmosphere of 5% CO_2 and 95% air. The number of cells per flask was 16.5×10^4, and the cell viability, as determined by the trypan blue dye exclusion method, was over 95% before and after 9 days of cell culture.

Effects of somatostatin or bromocriptine on PRL and GH release. After 3, 6, or 9 days of cell culture, the medium was discarded and 10^{-7} *M* of somatostatin (GH release inhibiting hormone; GH-IH, Sigma) or 10^{-7} *M* of bromocriptine (2-Br-α-ergocriptine; CB-154, Sandoz) dissolved in Ham's F-10 medium lacking serum (pH 7.7) was added to the DC cultures. Cells cultured in serum-free Ham's F-10 medium were used as controls. Five flasks were used for the control cultures or for the 10^{-7} *M* concentration of each agent, respectively. After 6 hr in the presence of these agents, the media were collected. These samples were kept frozen at −27° until assayed.

PRL and GH secretory responses after the removal of the agents. After the collection of the media mentioned above, 5 ml of Ham's F-10 medium lacking serum and agents were added to every flask, and the cells were again incubated for 6 hr to see if rebound increases in the hormones released would occur *in vitro*. The media were then collected and kept frozen until assay. In both experiments, the effects produced by the agents were compared to the results obtained in control cultures.

RIA of rat PRL and GH. Rat PRL was measured in duplicate by radioimmunoassay (RIA) methods previously described (8). Rat GH was also measured by double antibody RIA procedures using materials supplied by the NIAMDD. All samples were assayed in duplicate according to the procedures recommended by NIAMDD. Results were expressed in terms of the NIH standards, PRL-RP-1 and GH-RP-1. Intra- and interassay coefficients of variation were 2.1 and 4.8% for PRL, and 5.9 and 7.5% for GH, respectively.

Statistical analysis. The data were subjected to analysis of variance followed by the Student–Newman–Keul's test for comparing the differences between group means.

Results. *Effects of somatostatin or bromocriptine on PRL release.* The basal secretion rate of PRL increased progressively with increasing time in culture (Fig. 1A). At each of the three time periods not only bromocriptine but also somatostatin significantly suppressed the rate of PRL release compared to the control values ($P < 0.01$; Fig. 1A). When these data were expressed as a percentage of the control value (Fig. 1B) the inhibitory effect of somatostatin on PRL release was constant and varied from 26 to 29% at the three time periods. In contrast, the inhibitory effect of bromocriptine on PRL release was maximal on Day 3 (76% decrease from basal), and became attenuated at 6 days and at 9 days (52 and 32% decreases in PRL release from basal on Days 6 and 9, respectively). The inhibition by bromocriptine was far greater than that produced by somatostatin on Day 3 ($P < 0.01$) and on Day 6 ($P < 0.01$), but not on Day 9 (Fig. 1A).

PRL secretory responses after removal of the agents. After removal of the media containing the inhibitory agents the DC were reincubated for 6 hr in Ham's F-10 medium alone. Again the control flasks showed increases in PRL release with increasing time in culture and each value exceeded that of the former incubation (Fig.

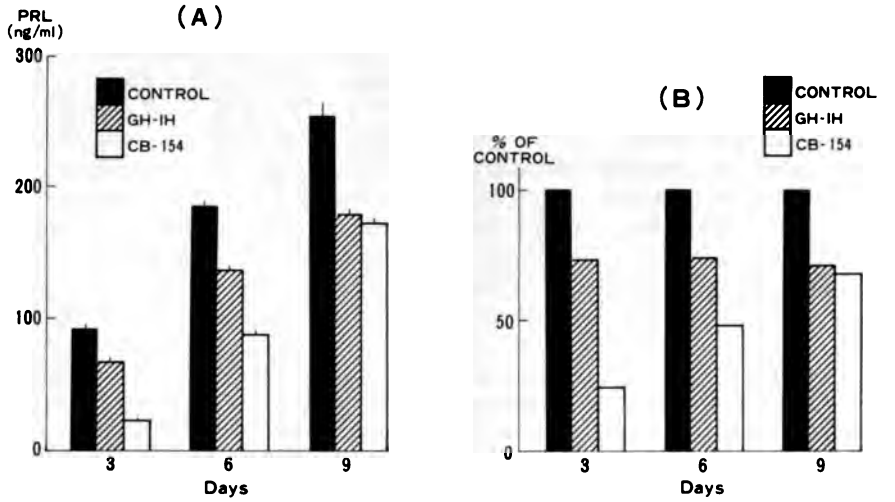

FIG. 1. (A) Effects of somatostatin (GH-IH) and bromocriptine (CB-154) on the release of PRL into Ham's F-10 culture medium during a 6-hr incubation. Each bar represents the mean ± SEM. (B) The mean values were expressed as percentage of control (100%).

2A). Even after the removal of the bromocriptine from the cultures the PRL inhibitory effect of this agent continued at almost the same degree as was seen with bromocriptine in the medium (71, 56, and 52% decrease from basal on Days 3, 6 and 9, respectively; $P < 0.01$ compared to control). In contrast, the removal of somatostatin from the media largely freed the cells from its inhibitory effect; only in the 9-day cultures was there still a significant inhibition of PRL release as compared to the control group ($P < 0.01$; Figs. 2A, B).

Effects of somatostatin or bromocriptine on GH release. Basal GH release rate increased with time in culture and reached a

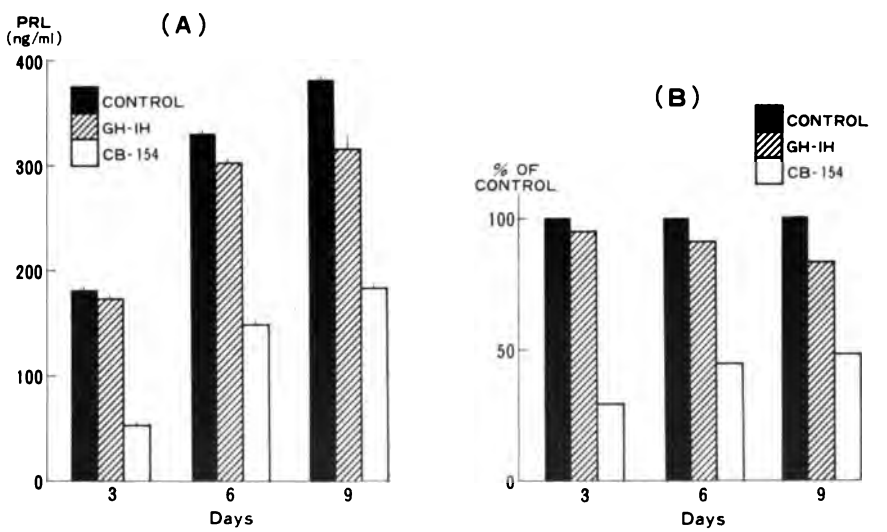

FIG. 2. (A) PRL secretory responses during 6-hr incubation with agent-free Ham's F-10 culture medium after the removal of somatostatin (GH-IH) and bromocriptine (CB-154). (B) See legend of Fig. 1B.

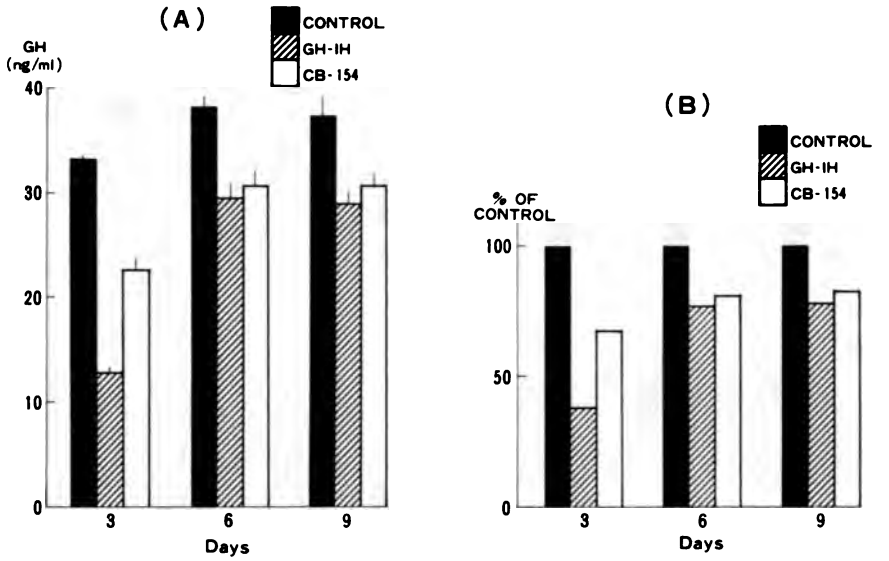

FIG. 3. (A) Effects of somatostatin (GH-IH) and bromocriptine (CB-154) on the release of GH into Ham's F-10 culture medium during a 6-hr incubation. (B) See legend of Fig. 1B.

plateau at 6 days (Fig. 3A). At each time, somatostatin and bromocriptine significantly inhibited the GH release compared to controls ($P < 0.05-0.01$). As shown in Fig. 3B, the inhibitory effects of somatostatin or bromocriptine on GH release were maximal on Day 3 (62 and 32% decrease, respectively) and were diminished on Day 6 (23 and 20% decrease) and on Day 9 (22 and 17% decrease, respectively). On Day 3, the inhibitory action of somatostatin was far greater than that of bromocriptine ($P < 0.01$). However, there were no significant differences between the inhibitory effects of somatostatin and bromocriptine on Days 6 or 9.

GH secretory responses after the removal of the agents. After removal of the inhibitory agents, the GH released in the control group was maximal on Day 3, and lessened with time although all of the basal control values were far greater than those seen in the first incubation (Fig. 4A). As with the PRL responses to bromocriptine, after the removal of somatostatin, the inhibitory effect on GH release persisted at a slightly higher level than that seen during the first incubation (67, 50, and 49% decrease on Days 3, 6, 9, respectively, $P <$ 0.01 compared to control; Figs. 4A, B). On the other hand, the cultures that had been treated with bromocriptine were freed from inhibition and showed no values which differed significantly from the controls (Figs. 4A, B).

Discussion. This study showed that bromocriptine or somatostatin can markedly suppress the rate of PRL and GH release from primary cultures of dispersed rat anterior pituitary cells. With increasing time in culture the PRL- or GH-inhibiting activity of bromocriptine or somatostatin, respectively, became somewhat decreased. Moreover, we observed no rebound increases in the release of either hormone after the removal of these inhibitory agents.

It has been reported that dopamine (DA) or DA agonists can suppress PRL and GH secretion in adult rats (9–15), although DA may have a stimulatory role on GH secretion in infant rats (16). In this study, bromocriptine had an inhibitory effect not only on PRL but also on GH release *in vitro*. Thus bromocriptine acts on pituitary mammotrophs and somatotrophs directly. Therefore, it seems possible that rat pituitary mammotrophs and somatotrophs both have receptors for bromocriptine. It has

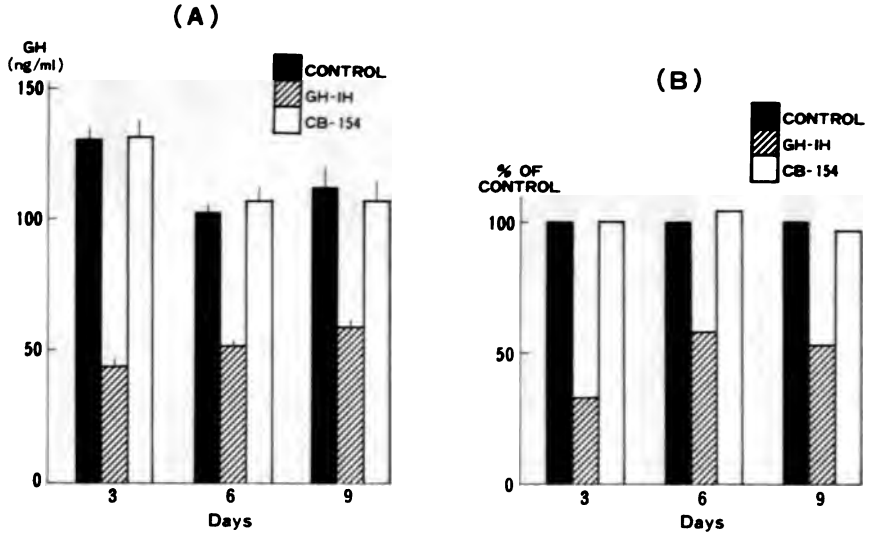

FIG. 4. (A) GH secretory responses during 6-hr incubation with agent-free Ham's F-10 culture medium after the removal of somatostatin (GH-IH) and bromocriptine (CB-154). (B) See legend of Fig. 1B.

been demonstrated that receptors for DA and apomorphine are both present in cells of the pituitary gland (17–19).

In rats, somatostatin suppresses GH secretion both *in vivo* and *in vitro*, while it inhibits PRL secretion only *in vitro* (20–22). Our results confirmed the *in vitro* effects of somatostatin on GH and PRL release. The reason somatostatin inhibits PRL release only *in vitro* is not known. One possibility may be that somatostatin can reveal this action only in the absence of hypothalamic regulation. In normal human subjects, somatostatin does not inhibit basal or TRH-induced PRL secretion (23, 24). However, it inhibits the basal levels of PRL in some acromegalic patients (25) and arginine-induced PRL release in normal men (26). In acromegalic patients, the properties of mammotrophs and the hypothalamic function of the tuberoinfundibular dopaminergic neurons may be different than in normal subjects (25, 27, 28).

Studies on the time course of the responsiveness of primary cultures of dispersed anterior pituitary cells to bromocriptine or somatostatin have not been previously reported. Bromocriptine had maximal inhibiting activites on PRL or GH release on Day 3, and this activity became lessened with time. Somatostatin caused a maximal inhibition of GH release on Day 3, and this inhibition decreased with time. In contrast, somatostatin showed a slight but stable inhibition of PRL release at each of the three times tested. Why bromocriptine and somatostatin had maximal inhibitory activities on PRL or GH release on Day 3, respectively, and then showed a lesser activity with increasing time in culture is not clear. These findings are at variance with the reports that surgical or pharmacological disruption of the hypothalamus causes supersensitivities of mammotrophs or somatotrophs to exogenous stimuli (29–31). However, in these experiments the pituitary glands were already manipulated *in vivo*, and the *in vitro* studies employed hemipituitary glands obtained immediately after decapitation. Therefore, the experimental conditions were quite different from ours and it is difficult to compare the results. Without the presence of receptor binding substances, it is possible that the number of receptors to regulating hormones and responsiveness of the cells will decrease, as has been suggested for patients with Addison's disease where

the high ACTH levels are not suppressed to normal ranges by glucocorticoids (32, 33).

Somatostatin and DA are important although not the only physiological regulators of GH and PRL secretion, respectively (9, 13, 20, 34). Bromocriptine is a potent DA agonist, and has been widely used as a substance to inhibit PRL release (13, 35). Although, the responsiveness of GH to somatostatin or PRL to bromocriptine changed markedly with time in our experiments those of GH to bromocriptine or PRL to somatostatin showed only slight changes. These findings may also suggest that, in the absence of hypothalamic control, somatotrophs and mammotrophs may lose their responsiveness to their major inhibiting agents, while the cells may retain their responsiveness to other less specific agents.

In vivo studies have shown that after the removal of inhibiting agents, PRL or GH are apt to show rebound increases exceeding the initial basal levels (1–7). We did not observe any rebound phenomenon in PRL or GH release after the removal of the inhibitory agents, although the level of hormones released from all the cultures, including the controls, was higher after the removal of somatostatin or bromocriptine. We believe these increases as well as the higher basal levels of hormone release must have been due to at least in part to the stimulatory effect of changing the culture media (36, 37).

There are several possible explanations for the mechanism of the post-inhibitory rebound seen *in vivo*. The rebound could be due to (1) increased hypothalamic releasing factors; (2) decreased hypothalamic inhibiting factors; or (3) an overshoot from the stored pituitary pool (3, 5). Our results mainly support the first or second explanations because without the hypothalamic control *in vitro* there were no rebound phenomena. These findings are similar to those of Martin *et al.* in which hypothalamic VMN (ventromedial nucleus) lesions in rats prevented the somatostatin-induced postinhibitory rebound (3).

In conclusion, rat pituitary mammotrophs and somatotrophs have been shown to respond *in vitro* to both bromocriptine and somatostatin. With the removal of hypothalamic control (i.e., *in vitro* system), these cells were most sensitive to these inhibitory agents on Day 3 in culture. Also, it is proposed that the post-treatment rebound increases exceeding basal levels in PRL and GH secretion seen *in vivo* must be mainly caused by hypothalamic modulations.

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1. Blake CA. Effects of intravenous infusion of catecholamines on rat plasma luteinizing hormone and prolactin concentrations. Endocrinology 98:99–104, 1976.
2. Levin JI, Voogt JL. Effects of dopamine, norepinephrine, and serotonin on plasma prolactin levels in ovariectomized, pituitary-grafted rats. Proc Soc Exp Biol Med 157:576–579, 1978.
3. Martin WH, Rogol AD, Kaiser DL, Thorner MO. Dopaminergic mechanisms and luteinizing hormone (LH) secretion. II. Differential effects of dopamine and bromocriptine on LH release in normal women. J Clin Endocrinol Metab 52:650–656, 1981.
4. Martin JB. Brain regulation of growth hormone secretion. In: Martini L, Ganong WF, eds. Frontiers in Neuroendocrinology. New York, Raven Press, p129, 1976.
5. Stachura ME. Influence of synthetic somatostatin upon growth hormone release from perifused rat pituitaries. Endocrinology 99:678–683, 1976.
6. Malven PV. Immediate release of prolactin and biphasic effects on growth hormone release following electrical stimulation of the median eminence. Endocrinology 97:808–815, 1975.
7. Leblanc H, Rigg LA, Yen SSC. The response of pancreatic and pituitary hormones to pulses and constant infusion of somatostatin. J Clin Endocrinol Metab 41:1105–1109, 1975.
8. Hanew K, Shiino M, Rennels EG. Effects of indoles, AVT, oxytocin, and AVP on prolactin secretion in rat pituitary clonal (2B8) cells. Proc Soc Exp Biol Med 164:257–261, 1980.
9. Gibbs DM, Neill JD. Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. Endocrinology 102:1895–1900, 1978.
10. Gudelsky GA, Porter JC. Release of newly synthesized dopamine into the hypophysial portal vasculature of the rat. Endocrinology 104:583–587, 1979.
11. Yeo T, Thorner MO, Jones A, Lowry PJ, Besser GM. The effect of dopamine, bromocriptine, lergotrile and metoclopramide on prolactin release from continuously perfused columns of isolated

rat pituitary cells. Clin Endocrinol 10:123–130, 1979.
12. Smalstig EB, Sawyer BD, Clemens JA. Inhibition of rat prolactin release by apomorphine in vivo and in vitro. Endocrinology 95:123–129, 1974.
13. MacLeod RM. Regulation of prolactin secretion. In: Martini L, Ganong WF, eds. Frontiers in Neuroendocrinology. New York, Raven Press, p169, 1976.
14. Collu R, Frashini F, Visconti P, Martini L. Adrenergic and serotonergic control of growth hormone secretion in adult male rats. Endocrinology 90:1231–1237, 1972.
15. Kokka N, Garcia JF, Elliott HW. Effects of acute and chronic administration of narcotic analgesics on growth hormone and corticotrophin (ACTH) secretion in rats. Prog Brain Res 39:347–360, 1973.
16. Müller EE, Nistico G, Scapagnini U. Neurotransmitters and Anterior Pituitary Function. New York, Academic Press, p246, 1977.
17. MacLeod RM, Kimura H, Login I. Inhibition of prolactin secretion by dopamine and piribedil (ET-495). In: Pecile A, Müller EE, eds. Growth Hormone and Related Peptides, Amsterdam, Excerpta Medica p443, 1976.
18. Caron MG, Beaulieu M, Raymond V, Gagné B, Drouin J, Lefkowitz RJ, Labrie F. Dopaminergic receptors in the anterior pituitary gland. J Biol Chem 253:2244–2253, 1978.
19. Cronin MJ, Robert JM, Weiner RI. Dopamine and dihydroergocriptine binding to the anterior pituitary and other brain areas of the rat and sheep. Endocrinology 103:302–309, 1978.
20. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179:77–79, 1973.
21. Vale W, Rivier C, Brazeau P, Guillemin R. Effects of somatostatin on the secretion of thyrotropin and prolactin. Endocrinology 95:968–977, 1974.
22. Drouin J, Léan AD, Rainville D, Lachance R, Labrie F. Characteristics of the interaction between thyrotropin-releasing hormone and somatostatin for thyrotropin and prolactin release. Endocrinology 98:514–521, 1976.
23. Siler TM, Vandenberg G, Yen SSC, Brazeau P, Vale W, Guillemin R. Inhibition of growth hormone release in humans by somatostatin. J Clin Endocrinol Metab 37:632–634, 1973.
24. Hall R, Besser GM, Schally AV, Coy DH, Evered C, Goldie DJ, Kastin AJ, McNeilly AS, Mortimer CH, Phenekos C, Tunbridge WMG, Weightman D. Action of growth-hormone-release inhibiting hormone in healthy men and in acromegaly. Lancet 2:581–584, 1973
25. Yen SSC, Siler TM, De Vane GW. Effect of somatostatin in patients with acromegaly. Suppression of growth hormone, prolactin, insulin and glucose levels. N Engl J Med 290:935–938, 1974.
26. Rodriguez-Arnao MD, Gomez-Pan A, Rainbow SJ, Woodhead S, Comaru-Schally AM, Schally AV, Meyers CA, Coy DH, Hall R. Effects of prosomatostatin on growth hormone and prolactin response to arginine in men. Comparison with somatostatin. Lancet 1:353–356, 1981.
27. Cryer PE, Daughaday WH, Growth hormone. In: Martini L, Besser GM, eds. Clinical Neuroendocrinology. New York, Academic Press, p243, 1977.
28. Collu R. Role of central cholinergic and aminergic neurotransmitters in the control of anterior pituitary hormone secretion. In: Martini L, GM Besser, eds. Clinical Neuroendocrinology. New York, Academic Press, p43, 1977.
29. Cheung CY, Weiner RI. In vitro supersensitivity of the anterior pituitary to dopamine inhibition of prolactin secretion. Endocrinology 102:1614–1620, 1978.
30. Anunziato L, Moore KE. Increased ability of apomorphine to reduce serum concentrations of prolactin in rats treated chronically with α-methyltyrosine. Life Sci 21:1845–1850, 1977.
31. Udeshini G, Cocchi D, Panerai AE, Gil-Ad I, Rossi GL, Chiodini PG, Liuzzi A, Müller EE. Stimulation of growth hormone release by thyrotropin-releasing hormone in the hypophysectomized rat bearing an ectopic pituitary. Endocrinology 98:807–814, 1976.
32. Holdaway IM. Suppression of plasma ACTH levels with corticosteroids in Addison's disease. Clin Endocrinol 2:37–42, 1973.
33. Krieger DT, Gewirtz GD. The nature of the circadian periodicity and suppressability of immunoreactive ACTH levels in Addison's disease. J Clin Endocrinol Metab 39:46–52, 1974.
34. Chihara K, Arimura A, K-Garfias C, Schally AV. Enhancement of immunoreactive somatostatin release into hypophysial portal blood by electrical stimulation of the preoptic area in the rat. Endocrinology 105:1416–1418, 1978.
35. Corrodi H, Fuxe K, Hökfelt T, Lidbrink P, Ungerstedt U. Effect of ergot drugs on central catecholamine neurons: Evidence for a stimulation of central dopaminergic neurons. J Pharm Pharmacol 25:409–411, 1973.
36. Groshong JC, Milo GE, Malarkey WB. The effect of aging on prolactin secretion in rat pituitary monolayer culture. Life Sci 20:1821–1828, 1977.
37. Kim KC, Burkman AM. Prolactin release from rat anterior pituitary cell culture following medium replacement. Proc Soc Exp Biol Med 167:551–553, 1981.

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The Transport of Urate in the Small Intestine of the Rat (41471)

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Abstract. The transport of urate in the small intestine of the rat was examined, *in vivo*, to determine if specific (mediated) transport systems are present. The secretory flux of urate was determined following the infusion of urate systemically, with or without oxanate, while the small intestine was perfused with an initially urate-free solution. The absorptive flux of urate was determined by perfusing the gut with solutions of known concentrations of urate. In some studies probenecid was infused systemically or added to the luminal perfusion solution. Over a wide range of concentrations of urate in the plasma or in the lumen, there was no evidence for saturation of either the secretory or absorptive fluxes of urate. Probenecid had no effect on either of the flux rates. These findings suggest that in the small intestine of the rat, the movement of urate out of or into the lumen occurs by passive diffusion and that, under the conditions of study, no evidence for facilitated transport can be demonstrated.

In previous studies from this laboratory, we have examined, in detail, the mechanisms subserving the excretion of urate by the kidney (1, 2). In the rat kidney, the transport of urate in both the reabsorptive and secretory directions are mediated transport processes (1, 2). Despite general agreement that the kidney is the major route of excretion of urate, there is ample data to indicate that the gastrointestinal tract also plays a role in the overall metabolism of urate (3). The nature of the transport of urate in the gastrointestinal tract, however, has not been clearly defined. In the present studies we have examined the rates of transfer of urate out of and into the small intestine of the rat in order to determine if specific transport systems for this organic anion are present.

Methods. Male Sprague–Dawley rats with free access to food and water prior to study were anesthetized with pentabarbital (50 mg/kg body wt injected intraperitoneally). A tracheotomy was performed, the femoral artery and vein were cannulated and the urinary bladder was catheterized. The abdominal cavity was opened via a midline incision and segments of the small intestine were cannulated with polyethylene catheters. Proximal segments of the small intestine were considered to be from the ligament of Trietz and extending distally 9 to 15 cm. Distal segments of the small intestine were considered to be 5 cm from the ileocecal valve and extending proximally 9 to 15 cm. Animals were placed on a heated board and body temperature was maintained at 37°. During the course of study each animal received an intravenous infusion of isotonic saline at a rate of 6 ml/hr. The small intestines were perfused at a rate of 12 ml/hr with a solution containing sodium 130 meq/liter, bicarbonate 30 meq/liter, potassium 5 meq/liter, and chloride 105 meq/liter. [^{3}H]Polyethylene glycol (PEG) (New England Nuclear Corp., Boston, Mass.) was added as a volume marker. The collection periods were 10 to 20 min in duration.

In 30 animals the secretory flux of urate was determined by the systemic infusion of urate with or without oxanate, an inhibitor of uricase, while perfusing the intestine with an initially urate free solution. In order to obtain a range of plasma concentrations of urate, sodium urate in concentrations of 10, 25, or 50 mg% and oxanate in concentrations of 0.5 or 1.0 g% were added to the

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intravenous infusion solution. Where examined, probenecid was also added to intravenous infusion solution in concentrations calculated to deliver a dose equal to 100 mg/kg body wt/hr. In other studies, probenecid (28.5 mg%) was added to the intestinal perfusion solution.

In the studies examining the absorptive flux of urate, no urate or oxanate was infused systemically (20 animals). Sodium urate was added to the intestinal perfusion solution in concentrations of 5 to 20 mg%. Where examined, probenecid was infused systemically or added to the luminal perfusion solution in the amounts previously indicated.

In both the secretory and absorptive flux studies, two intestinal perfusions were obtained at any given intravenous infusion rate or any given initial concentration of urate in the perfusion solution. The urate concentrations were then changed, 20 additional min allowed to elapse to allow for a new steady state and additional collections obtained. Usually three such periods at different concentrations of urate were obtained per animal. At the end of the experiments, the distance between the perfusion and collection intestinal catheters was measured. The average length of perfused segments of the small intestine averaged 11.6 ± 0.05 cm for the proximal segments and 11.4 ± 0.61 cm for the distal segments.

The radioactivity of perfusion and collection solutions was determined in Biofluor (New England Nuclear Corp.) in a liquid scintillation counter. The concentration of urate was determined by high performance liquid chromatography with electrochemical detection as previously described (4). Neither oxanate or probenecid interfered with the determination of the concentrations of urate.

The *in vivo* perfusion rate was calculated from the formula: perfusion rate (ml/hr) = collected volume (ml/hr) × CF/PF_{PEG} where CF and PF are the disintegrations per minute of PEG in the collected fluid (CF) and perfusion solution (PF), respectively. The secretory flux of urate was calculated from

$$J_s \text{ (g/min/cm)} = \text{CF urate} \times \text{collected volume} \times \text{length}^{-1},$$

where CF urate is the concentration of urate in the collected perfusion solution, and length is the distance between perfusion and collection sites in centimeters. The absorptive flux was calculated from

$$J_a \text{ (g/min/cm)} = \{(1\text{-}(CF/PF \text{ urate})/(CF/PF_{PEG})\} \times \text{perfusion rate} \times \text{PF urate} \times \text{length}^{-1}$$

where PF urate is the concentration of urate in the inital perfusion solution. The results of the duplicate collections were averaged and the results presented as the mean of means ± SEM.

Results. The CF/PF_{PEG}, an index of water absorption averaged 1.04 ± 0.004 and 1.06 ± 0.006 for proximal and distal segments, respectively. The perfusion rates were also similar in both intestinal segments and averaged 12.4 ± 0.09 ml/hr for proximal segments and 13.4 ± 0.21 ml/hr for distal segments. The rates or water absorption were not effected by the presence of urate or probenecid in the lumen or by the systemic infusion of urate or probenecid.

For the sake of data presentation, the fluxes are averaged for urate concentrations over a range of 1 mg% for plasma urate concentrations from less than 1 to 5 mg% and in a 3 mg% intervals for higher plasma concentrations of urate. There were no significant differences in the rates of urate secretion in proximal and distal intestinal segments at any given plasma concentration of urate. In addition, neither the systemic infusion of probenecid nor the inclusion of probenecid in the luminal perfusion solution had any effect on the secretion of urate. The results of all secretory studies were combined and are plotted as a function of the plasma concentration of urate (Fig. 1). As can be seen, over the wide range of plasma concentrations of urate obtained, the secretion of urate appeared to be a direct function of the plasma concentration and no evidence for saturation of the secretory flux is evident.

The absorptive flux of urate was also similar in both proximal and distal segments and was not influenced by the inclusion of probenecid in the perfusion solution. Figure

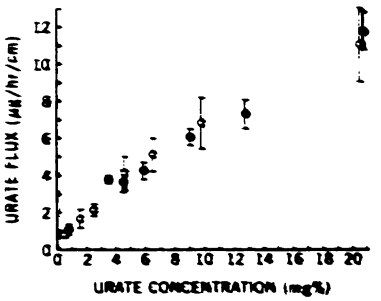

FIG. 1. The relationship between the secretory flux of urate (closed circles) or the absorptive flux of urate (open circles) and either the plasma concentration of urate in the case of the secretory studies or the arithmetic mean intraluminal concentration of urate in the absorptive studies. Values represent mean of means ± SEM. Each point is the average of 4 to 15 experimental periods.

1 summarizes the results of these studies and illustrates the absorptive flux as a function of the arithmetic mean intraluminal concentration of urate. In these studies, the plasma concentration of urate was less than 1 mg%. The values illustrated were grouped in a manner identical to that of the secretory studies. As was the case in the secretory studies, the absorptive flux is a direct function of the mean intraluminal concentration of urate and no evidence for saturation of the absorptive flux is evident. At any given concentration of urate, the absorptive flux approximates the secretory flux.

Discussion. It is known that the ingestion of a high purine diet increases the urinary excretion of urate and, in some individuals, exacerbates hyperurecemia. This response presumably reflects absorption of urate precursors which are metabolized to urate in the liver. It has also been demonstrated that urate itself can be reabsorbed from the gastrointestinal tract (3). The question of gut absorption of urate might be rendered moot when it is appreciated that diet of neither man nor rat contains any preformed urate. On the other hand, the gut is thought to dispose of 25 to 33% of the daily production of urate (3). Reabsorption of this component of urate in the lumen could be of potential importance. The mechanism whereby urate enters the intestine has not been clarified. Conceivably, urate could enter as components of gastrointestinal secretions, passively diffuse from the blood into the gastrointestinal tract, or be secreted by specific mediated transport systems analogous to those present in the kidney (1, 2).

Kolassa *et al.* have recently presented evidence that hypoxanthine and xanthine are actively secreted by the jejunum of the guinea pig (5). The transport of urate itself, however, was not investigated in these studies. Berlin and Hawkins have also reported active secretion of hypoxanthine and xanthine in the small intestine of the golden hamster (16). Although less definitive than for hypoxanthine and xanthine, these studies also suggested that the carrier had specificity for urate as well. Scharrer *et al.* have demonstrated an active absorptive flux for hypoxanthine in the jejunum of the lamb (7). The transport of urate was not specifically examined in these studies but urate had no effect on the transport of hypoxanthine. Active absorption of some purines and pyrimidines has also been demonstrated in the chiton but no evidence for the active absorption of urate was found in this species (8).

Shanker *et al.* demonstrated that the rat intestine was capable of active absorption of uracil and that certain purine and purine-like compounds could inhibit its transport (9). Although it was not possible to demonstrate unequivocally that urate itself was transported, urate did inhibit, albeit weakly, the transport of uracil suggesting a common transport mechanism. Wilson and Wilson, Kahn *et al.*, and Oh *et al.*, however, have all presented evidence that urate transport across the small intestine of the rat was by passive diffusion only and no active or mediated transport systems could be discerned (10–12). The validity of the conclusions of these studies has been questioned in some of these experiments since the studies were performed *in vitro* and no data were presented to indicate that the tissues examined were capable of active transport of other substrates (5). Moreover, these studies employed [2-^{14}C]urate which can conceivably undergo

metabolic transformation to radioactive allantoin.

Since some uncertainty as to whether or not the intestine of the rat is capable of transport of urate, and since the intestine may be important in the overall disposition of urate, we elected to reinvestigate this question using the techniques of *in vivo* intestinal perfusion. In the rat, urate is converted to allantoin and, as a consequence of this conversion, the infusion of urate systemically does not lead to predictable elevations in the serum concentration of urate. Moreover, renal function was intact in our animals and no doubt the urinary excretion of urate was increased. However, by infusing varying amounts of urate with or without oxanate, a hepatic inhibitor of uricase, we were able to study the recovery of urate in perfused segments of the gut in response to a wide range of plasma concentrations. The secretory flux of urate into the small intestine, at any given plasma concentration of urate, was similar in proximal and distal segments of the small intestine. As summarized in Fig. 1, no clear evidence of saturation of the secretory process was evident although some flattening of the relationship is noted at the highest plasma concentrations of urate obtained. It might be argued that a low capacity transport system does exist and was nearly saturated even at the lowest concentrations examined. It is also possible that a carrier system is present, one which has a K_m above that of the plasma concentrations obtained in the present study. Against these suggestions is the fact that probenecid in concentrations or amounts known to inhibit the renal transport of urate, had no influence on transport in the small intestine. To the degree that probenecid is a reasonably specific inhibitor of organic anion transport in a variety of tissues and based upon the relationship between the secretory flux and the plasma concentration, it seems reasonable to conclude that urate enters the lumen of the intestine by a passive mechanism and that a specific mediated transport system cannot unequivocally be demonstrated. We have also investigated the absorptive flux of urate over a range of concentrations of urate in the lumen. The imposition of a lumen to blood gradient for urate results in absorption of luminal urate. There was a direct relationship between the average luminal concentration and the absorptive flux over a wide range of urate concentrations. There was no clear evidence however for saturation of the absorptive process. Moreover, as in the secretory studies, the absorptive flux of urate was not influenced by the infusion of probenecid systemically or the inclusion of probenecid in the luminal solution. Using analogous considerations to those already discussed, it seems likely that the absorption of urate is also occurring by passive permeation only. The conclusions about both the nature of the secretory and the absorptive flux are entirely consistent with some of the evidence already cited from studies performed in the rat (10–12).

It is possible that species differences account for some of the disparities in the literature and it is recognized that interspecies variability in the renal handling of organic anions exists. It is likely that such may also exist in the intestinal transport of organic anions. It seems reasonable to conclude from the present studies plus the studies performed by others that in the small intestine of the rat, urate transport is most likely due to passive diffusion. The present studies, however, do not rule out the possibility of mediated transport of urate in the terminal portion of the ileum.

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1. Weinman EJ, Sansom SC, Steplock DA, Sheth AV, Knight TF, Senekjian HO. The secretion of urate in the proximal convoluted tubule of the rat. Amer J Physiol 239 (Renal Fluid Electrolyte Physiol 8):F383–F387, 1980.
2. Sansom SC, Senekjian HO, Knight TF, Babino H, Steplock DA, Weinman EJ. Determination of the apparent transport constants for urate absorption in the rat proximal tubule. Amer J Physiol **240** (Renal Fluid Electrolyte Physiol 9):F406–F410, 1981.
3. Sorensen LB. The elimination of uric acid in man. Scand J Clin Lab Invest Suppl 54:1–214, 1960.
4. Weinman EJ, Sansom SC, Steplock DA, Sheth AV, Knight TF, Senekjian HO. The use of high per-

formance liquid chromatography for determination of urate concentrations in nanoliter quantities of fluid. Kidney Int 19:81–85, 1981.
5. Kolassa N, Schutzenberger WG, Weiner H, Turnheim K. Active secretion of hypoxanthine and xanthine by guinea pig jejunum in-vitro. Amer J Physiol 238 (Gastrointest Liver Physiol 1):G141–G149, 1980.
6. Berlin RD, Hawkins RA. Secretion of purines by the small intestine: Transport mechanism. Amer J Physiol 215:942–950, 1968.
7. Scharrer E, Raab W, Tiemeyer W, Amann B. Active absorption of hypoxanthine by lamb jejunum in-vitro. Pflugers Arch 391:41–43, 1981.
8. Hanisch ME, Lawrence AL. Purine and pyrimidine absorption by the gut of the chiton, cryptochiton Stelleri. Comp Biochem Physiol 42A:601–610, 1975.
9. Shanker LS, Jeffrey JL, Tocco DJ. Interaction of purines with the pyrimidine transport process of the small intestine. Biochem Pharmacol 12:1047–1053, 1963.
10. Wilson DW, Wilson HCW. Studies in-vitro of the digestion and absorption of purine ribonucleotides by the intestine. J Biol Chem 237:1643–1647, 1962.
11. Kahn AM, Wilson S, Crawhall J. The influx of uric acid and other purines into overted jejunal sacs of the rat and hamster. Canad J Pharmacol 53:113–119, 1975.
12. Oh JH, Dossetor JB, Beck IT. Kinetics of uric acid transport and its production in rat small intestine. Canad J Physiol Pharmacol 45:121–127, 1967.

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Phosphoenolypyruvate (PEP) Effects on Fresh and Stored Red Blood Cells[1,2] (41472)

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Abstract. The present studies were performed to evaluate the effects of phosphoenolpyruvate (PEP) on fresh and stored red blood cells as a possible adjunct to citrate phosphate dextrose (CPD) for preservation of blood. Red cell concentrates prepared from fresh blood drawn from human volunteers were incubated with CPD alone or CPD containing PEP. At 37° and an initial pH of less than 6.2, a significant increase in 2,3-DPG and maintenance of ATP were observed. A dose-dependent increase in 2,3-DPG was noted in blood incubated in CPD–PEP from 13 to 52 m*M*. 2,3-DPG did not change during incubation at either 4° or 25°. A significant increase in ATP was observed at 25°; ATP remained unchanged at 4° and 37°. Storage related depletion of 2,3-DPG and ATP was reversed by PEP incubation (26 m*M*) even when blood was stored for 42 days in CPD. In fact, 2,3-DPG levels two or three times greater than normal were regularly observed. The persistence, *in vivo*, of the PEP-induced increase in 2,3-DPG and P_{50} was demonstrated by 33% exchange transfusions in rats with homologous blood treated for 4 hr with 26 m*M* PEP. Unlike other preservation additives, incubation with PEP results in a dramatic increase in 2,3-DPG without depletion of ATP, a property which is maintained *in vivo*. Evidence shows that PEP should be considered as a potential adjunct to conventional blood preservation systems.

Tomoda *et al.* (1) and Hamasaki *et al.* (2–4) have demonstrated that the glycolytic intermediate phosphoenolpyruvate (PEP) accumulates inside human erythrocytes which have been incubated in acidified sucrose and citrate solutions containing PEP. Transport of PEP across the red cell membrane and its subsequent metabolism results in a significant increase in the intracellular concentration of 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP) during incubation in these media (1–4).

The present studies were designed to assess the utility of PEP as an adjunct to (a presently available blood preservation system) citrate phosphate dextrose (CPD). The importance of 2,3-DPG and ATP as *in vitro* indicators of erythrocyte function and viability suggests that a treatment which results in their simultaneous generation or maintenance would significantly improve our ability to store blood in the liquid state. We have evaluated the effects of varying concentrations of PEP on both fresh and stored red blood cells. Time, temperature, and pH of incubation were varied to determine optimum conditions for maximal generation of 2,3-DPG and maintenance of ATP.

Materials and Methods. *Fresh blood.* Fresh blood was drawn from healthy volunteers into syringes which had been prefilled with standard citrate phosphate dextrose (CPD) solution to achieve a final blood–anticoagulant mixture ratio of 7:1.[3,4]

[1] Presented in part at the American Federation of Clinical Research, Western Section Meeting, February 5, 1981, Carmel, Calif.

[2] The opinions or assertions contained herein are private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense (AR 360–5).

[3] Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70–25 and USAMRDC Reg 50–25 on the use of volunteers in research.

[4] The citation of trade names in this report (or paper) does not constitute an official endorsement or approval of the use of such items.

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Red cell concentrates (RCC) were prepared after centrifugation in a Sorvall RC-3 refrigerated centrifuge at 5000*g* for 5 min at 4°. Aliquots (4 ml) of the resultant RCC were incubated at 37° for 5 min in a shaking water bath (rpm = 140). Equal volumes of test solutions were added and samples removed for measurement of hemoglobin, 2,3-DPG, ATP, pH, and P_{50}. The aliquots were then incubated at 4°, 25°, or 37° for 4 hr. Samples were removed at intervals for the biochemical analyses noted above. Test solutions were prepared by dissolving phosphoenolpyruvate (tricyclohexylammonium salt obtained from Sigma Chemical Co., St. Louis, Mo.) in sterile CPD; the pH was adjusted to 5.7 with dropwise addition of 0.1 *M* NaOH or 0.1 *M* HCl.

The first experiment was designed to evaluate the effects of the concentration of PEP on P_{50} and intraerythrocytic 2,3-DPG and ATP. Test solutions were prepared to achieve final concentrations (test solution plus RCC) of 0, 13, 26, 52, and 78 m*M* PEP. After mixing and initial sampling, the RCC test solution mixtures were incubated at 37° for 4 hr. Samples for analysis of P_{50}, 2,3-DPG, and ATP were removed after 1 and 4 hr of incubation.

The second experiment was designed to evaluate the effects of incubation temperature of P_{50} and intraerythrocytic 2,3-DPG and ATP. Equal volumes of CPD or 52 m*M* PEP in CPD were added to red cell concentrates. The PEP and CPD–RCC mixtures were incubated at 4°, 25°, and 37° for 4 hr of incubation.

The third experiment was designed to evaluate the effects of pH on intraerythrocytic 2,3-DPG and ATP. Equal volumes of CPD or 52 m*M* PEP in CPD solution were added to red cell concentrates. The pH of the RCC solution mixtures was adjusted to 6.2, 6.6, and 7.2 (pH measures at 37°) by dropwise addition of 1 *M* NaOH or 0.1 *M* HCl. The RCC solution mixtures were incubated at 37° for 4 hr. Samples were removed for biochemical analysis after 1 and 4 hr of incubation.

In the fourth experiment, rats were subjected to a 33% exchange transfusion with either PEP-treated or control rat red blood cells to determine the *in vivo* persistence of the high 2,3-DPG and P_{50} induced by PEP incubation. Fresh rat blood was collected in CPD (1:7, V:V) from 16 retired breeder rats, 600–800 g. The donor rats were anesthetized with phenobarbital 50 mg/kg (Nembutal, Sodium, Abbott Lab., Chicago, Ill.) and bled via the abdominal aorta. Blood from two rats was pooled for each transfusion. Four pools were incubated with PEP for 4 hr as previously described (5) and four pools were held in the refrigerator at 4° for 4 hr. Following incubation or refrigeration, the red cells were washed twice in three vol of acid saline and resuspended in warm 4% albumin in 0.9% saline solution. The pH of the PEP-treated suspensions was 7.369 ± 002, the P_{50} was 56.6 ± 4.7, and the 2,3-DPG content was 31.45 ± 3.18 μm/g Hgb. The pH of the control suspension was 7.380 ± 0.011, the P_{50} was 38.6 ± 0.7, and the 2,3-DPG content was 2.324 ± 1.19 μm/g Hgb. Male Sprague–Dawley rats, 250–300 g, were used as recipients. The animals were anesthetized as above, and the right exterior carotid artery and jugular vein were catheterized with PE-50 polyethelene and silastic (0.04 in 1.0) catheters, respectively. One-third of the total blood volume was exchanged by withdrawal of 2 ml/100 g body weight through the carotid cannula and replacement with an equal volume of cell suspension through the jugular catheter. Following transfusion, the jugular catheter was removed, and the carotid cannula was tunnled subcutaneously to the back of the neck and exteriorized between the scapulae. The animals were then allowed to recover, and post-transfusion samples were drawn through the carotid cannula 6 hr following the transfusion.

Stored blood. A total of 450 ml of blood was drawn from each of three healthy volunteers and collected into McGaw Haemo-Pak units containing 63 ml of CPD. These units were stored as whole blood (Hct = 40 ± 5) under standard blood bank conditions at 4°. Aliquots (10 ml) were removed at weekly intervals and placed on ice. Baseline P_{50}, ATP, and 2,3-DPG levels were measured. The remaining sample from each unit was divided equally and incubated at

37°, pH 5.9–6.2, for 4 hr in equivalent volumes of CPD or PEP in CPD. The final concentrations of PEP in the experimental blood–solution mixtures was 26 m*M*. The P_{50}, ATP, and 2,3-DPG levels were measured immediately after mixing and after 1 and 4 hr of incubation in a shaking 37° water bath (rpm = 140) with either control (CPD) or experimental (PEP in CPD) solution.

Analytical procedures. P_{50} was measured by the biotonometry method described by Neville (5). Concentrations of ATP and 2,3-DPG were determined from protein-free filtrates prepared with 6% perchloric acid (0.5:2.0, blood:PCA) neutralized with potassium bicarbonate. Assays were performed using commercially available assay kits (ATP, Sigma technical bulletin 366-UV; 2,3-DPG, Sigma technical bulletin 35-UV). Glucose was measured in the protein-free filtrates using a hexokinase technique (7).

Results. *Fresh blood.* The effects of varying the concentration of PEP on intraerythrocytic 2,3-DPG are presented in Fig. 1. A progressive fall in 2,3-DPG occurred in CPD alone such that levels 25% of the initial value were observed after incubation for 4 hr. Increasing the concentration of PEP from 13 to 52 m*M* resulted in increased generation of 2,3-DPG. Postincubation 2,3-DPG levels obtained with 78 m*M* PEP did not differ from those obtained with a 52 m*M* solution. The effect of varying the concentration of PEP on pH, P_{50}, and ATP are presented in Table I. A progressive fall in ATP occurred in CPD alone; an increment in ATP was observed with all concentrations of PEP. Increments in P_{50} paralleled the rise in 2,3-DPG. An initial increase in P_{50} was observed following addition of CPD alone. This could be attributed to an alkaline Bohr effect due to the low pH of the incubation mixture (8). However, the P_{50} increments observed with PEP incubation were significantly greater ($P < 0.05$) than those achieved with CPD alone, although the pH was the same in both CPD and PEP incubations. A moderate degree of hemolysis was observed when PEP was added to exceed a final concentration of 26 m*M*.

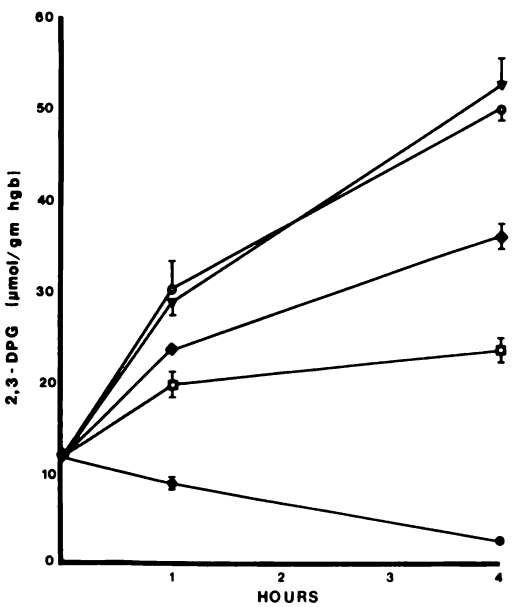

FIG. 1. Effect of varying PEP concentration on red cell 2,3-DPG production. Each value represents mean ± SEM (n = 3) symbols without vertical bars indicate the standard errors were smaller than the symbol used. ▽, 78 m*M*; ○, 52 m*M*; ◆, 26 m*M*; □, 13 m*M*; ●, 0 m*M*.

The effect of incubation temperature on intraerythrocytic 2,3-DPG is presented in Fig. 2. A 26 m*M* concentration of PEP in CPD was chosen because of hemolysis observed with higher concentrations. A dramatic increase in 2,3-DPG was observed at 37°. When measured at 4 hr, 2,3-DPG was three times greater than initial value. 2,3-DPG was maintained without significant change at 4° in both PEP–CPD and CPD alone. When the blood–PEP in CPD mixture was incubated at 25°, 2,3-DPG was also maintained at a level which approximated the initial value, whereas incubation at 25° with CPD alone resulted in a slight reduction in 2,3-DPG concentration. The effects of incubation temperature on ATP are presented in Fig. 3. ATP was maintained close to its initial value in PEP in CPD (and CPD alone) at 4° after incubation for 4 hr. However, a small but significant increase ($P < 0.05$) in ATP was observed at 25°. ATP was maintained close to its initial value at 37°.

The effect incubation pH on intraerythrocytic 2,3-DPG and ATP is presented in Table II. A fall in 2,3-DPG concentration

TABLE I. EFFECT OF VARYING PEP CONCENTRATION ON RED CELLS

		1 hr				
	Initial	0 m*M*	13 m*M*	26 m*M*	52 m*M*	78 m*M*
ATP[1,2] (μmol/g Hgb)	3.72 ± .16	3.55 ± .14	4.09 ± .51	4.35 ± .58	4.50 ± .69	4.06 ± .24
pH[1,2]	7,065 ± .038	6.25 ± .008[b,a]	6.287 ± .013[a]	6.196 ± .024[b]	6.088 ± .034[c]	6.057 ± .019[c]
P_{50}[1,2]	25.4 ± .61	34.55 ± 1.65	39.08 ± .87	38.79 ± 1.03	38.04 ± .60	37.47 ± 1.63

Table 1. *(cont'd).*

		4 hr				
	Initial	0 m*M*	13 m*M*	26 m*M*	52 m*M*	78 m*M*
ATP[1,2] (μmol/g Hgb)	3.72 ± .16	2.81 ± .05[b]	3.81 ± .21[a]	4.09 ± .28[a]	4.31 ± .08[a]	3.99 ± .74[a]
pH[1,2]	7,065 ± .038	6.347 ± .019[a]	6.376 ± .024[a]	6.321 ± .031[a]	6.203 ± .041[b]	6.126 ± .010[c]
P_{50}[1,2]	25.4 ± .61	30.89 ± 99[b]	42.93 ± .74[a]	44.24 ± 1.06[a]	46.95 ± 1.30[a]	45.07 ± .23[a]

[1] Each value represents mean ± SEM, $n = 3$.

[2] Analysis of variance was performed within each time period. If a F test indicated a significant difference ($P < 0.05$) existed, Duncans multiple range test was performed. Means with same superscripts are not significantly different ($P < 0.05$). Means with no superscripts indicate an insignificant F statistic was calculated.

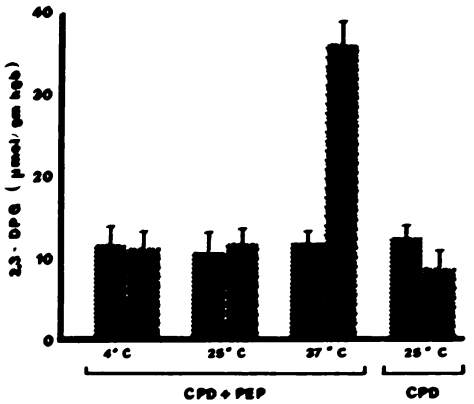

FIG. 2. Effect of temperature of incubation on red cell 2,3-DPG. At each temperature tested, the left bar represents initial mean 2,3-DPG; the right bar represents 2,3-DPG measured after 4 hr of incubation. Vertical bars represent SEM (n = 4). A paired t test was used to determine if the 2,3-DPG concentrations were different before and after incubation at each temperature. There was a significant ($P < 0.001$) increase when cells were incubated at 37° in the presence of PEP. There was a significant ($P < 0.001$) decrease when cells were incubated at 25° in CPD alone.

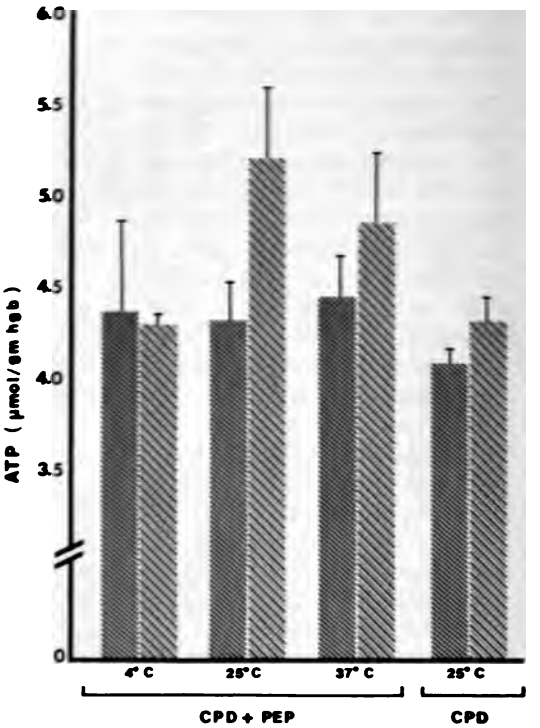

FIG. 3. Effect of temperature of incubation on red cell ATP. At each temperature examined, the left bar represents initial mean ATP, the right bar represents ATP after 4 hr of incubation. Vertical bars represent the SEM (n = 4). A paired t list was used to determine if the ATP concentration after incubation differed from the initial value. There was a significant increase ($P < 0.05$) in ATP concentration only when cells were incubated in the presence of PEP at 25°.

which was similar to that observed with CPD alone occurred at both pH 6.7 and 7.2 in red cells which had been incubated in PEP in CPD. However, incubation at pH 6.2 resulted in a 100% increase in 2,3-DPG. ATP concentration increased or was maintained regardless of the pH of incubation with PEP in CPD whereas, with CPD alone it was maintained at pH 6.7 and 7.2, but decreased at pH 6.2.

Stored blood. The effect of storage and subsequent incubation with either CPD or PEP in CPD on P_{50} is presented in Table III. As expected, P_{50} decrease progressively relative to the length of time in storage in CPD from Day 14 to Day 42. On all days studied, post-storage incubation with CPD alone resulted in an initial increase in P_{50} which can be attributed to the reduced pH of the incubation medium and an alkaline Bohr effect. Incubation with PEP in CPD resulted in P_{50} values which were significantly greater than that achieved with CPD alone. This effect was noted on all days studied.

The effect of storage and subsequent incubation with either CPD or PEP in CPD on red cell 2,3-DPG is presented in Table IV. As expected, red cell 2,3-DPG decreased progressively during storage in CPD. Post-storage incubation with CPD alone resulted in a further decrement in 2,3-DPG on all days studied. However, incubation with PEP in CPD resulted in a significant increase in 2,3-DPG; levels two to three times greater than those found in fresh red blood cells before storage were observed even after 42 days of *in vitro* storage.

The effect of storage and subsequent incubation with either CPD or PEP in CPD on red cell ATP is presented in Table V. Post-storage incubation with CPD alone resulted in a reduction in intraerythrocytic ATP.

TABLE II. EFFECT OF pH OF INCUBATION ON RED BLOOD CELLS INCUBATED WITH PEP

	Fresh blood	Post-4-hr incubation					
		CPD			CPD + PEP		
		6.2	6.7	7.2	6.2	6.7	7.2
2,3-DPG[2,3] (μmol/g Hgb)	13.03 ± 1.70[c]	4.69 ± 0.73[a]	5.07 ± 1.20[a]	8.76 ± 0.01[b]	26.22 ± 0.83[d]	8.13 ± 0.71[b]	11.71 ± 0.82[c]
ATP[2,3] (μmol/g Hgb)	4.00 ± 0.10[b,c]	3.00 ± 0.00[a]	3.80 ± 0.20[b]	4.35 ± 0.05[c,d]	4.45 ± 0.05[d]	3.95 ± 0.15[b,c]	4.75 ± 0.15[d]

[1] pH.
[2] Each value represents mean ± SEM, $n = 4$.
[3] Analysis of variance was performed initially; Duncan's multiple range test was performed to determine which means differed. Means with same superscripts are not significantly different ($P < 0.05$).

However, incubation with PEP in CPD resulted in a slight increment or maintenance of ATP on all days studied.

The *in vivo* effects of 33% exchange transfusion with PEP treated or control rat red cells are presented in Table VI. Although only one-third of the blood was replaced, there was a significant increase in P_{50} following transfusion. This result indicates (a) the cells are viable and are not cleared by 6 hr post-transfusion and (b) that there is no immediate catabolism of the supranormal 2,3-DPG levels following transfusion.

Discussion. Since Chanutin and Curnish (9) and Benesch and Benesch (10) first observed that the interaction of 2,3-DPG with hemoglobin reduces hemoglobin-oxygen affinity, the importance of 2,3-DPG as a mediator of oxygen delivery has been confirmed both *in vitro* and *in vivo* (11). A functional red cell defect reflected by an increase in hemoglobin-oxygen affinity has been directly correlated with depletion of 2,3-DPG in all conventional liquid blood preservation systems. A variety of additives have been employed to maintain red cell 2,3-DPG and hemoglobin function during prolonged liquid blood storage. In general, these additives have stimulated increased generation of 2,3-DPG at the expense of the ATP (12–14) whose contribution to the maintenance of red cell membrane integrity (15, 16), deformability (17), and post-transfusion viability (18, 19) is well accepted. Thus, hemoglobin function may be preserved at the expense of red cell viability. Conversely, additives such as adenine, which stimulate generation of ATP do so at the expense of 2,3-DPG (20); thus, red cell viability may be preserved at the expense of function.

In the present studies, we have confirmed the earlier reports by Tomoda *et al.* (1) and Hamasaki *et al.* (2–4), regarding incubation of human red blood cells in a citrate solution which contains PEP. In addition, we have demonstrated that the incubation of red blood cells stored for long periods in CPD with PEP results in the generation of 2,3-DPG and the simultaneous generation of ATP. Unlike other preservation additives, PEP may facilitate the mainte-

TABLE III. EFFECT OF INCUBATION WITH PEP ON P_{50} OF RED CELLS STORED IN CPD

		P_{50} (mm Hg)			
		1 hr[1,2]		4 hr[1,2]	
Day	Initial	CPD	PEP	CPD	PEP
0	25.52 ± 1.03	33.24 ± 2.41	40.95 ± 2.49	34.16 ± 3.44	48.63* ± 1.43
7	24.45 ± 3.26	33.36 ± 3.34	39.67 ± 2.56	35.64 ± 3.8	48.74* ± 2.31
14	20.31 ± 2.30	28.39 ± 1.67	39.44* ± 1.40	33.80 ± .12	49.05* ± .73
21	17.85 ± .65	26.04 ± 1.10	38.96* ± .75	34.01 ± 2.18	52.25* ± 1.53
28	18.07 ± .36	25.74 ± .04	39.82* ± .62	34.47 ± .52	56.77* ± 2.67
35	18.75 ± .71	28.00 ± .98	41.22* ± 1.29	36.82 ± 2.19	60.62* ± 2.67
42	17.89 ± 1.16	24.37 ± 1.35	38.52* ± 2.84	29.90 ± 2.05	53.03* ± 6.66

[1] Each value represents mean ± SEM; $n = 3$.
[2] On each sampling day, comparisons were made between cells incubated in CPD alone and CPD + PEP at each time period; an asterisk (*) indicates a significant difference was found using a paired t test ($P < 0.05$).

nance of *both* hemoglobin function and red cell viability during or after (rejuvenation) prolonged blood storage. However, the results of our efforts to optimize the PEP treatment clearly demonstrate that its effectiveness is dependent on the maintenance of incubation conditions which are generally considered to be detrimental to human red blood cells.

Tomoda *et al.* (1) and Hamasaki *et al.* (2–4) have demonstrated that PEP accumulates inside human erythrocytes which have been incubated in acidified sucrose and citrate solutions (pH = 4.5–6.5). Transport is apparently mediated via a membrane-bound anion transport system. In the presence of solutes to which the red cell membrane is impermeable (sucrose and citrate) the Donnan equilibrium is maintained such that PEP can accumulate within the red cell against the concentration gradient (2). The present studies confirm the pH dependency of the PEP transport. We have observed that the optimum pH range is 5.9 to 6.2 using standard CPD solution as the PEP solvent. Above this range, PEP has no effect on incubated red blood cells.

The effect of PEP on P_{50} is negligible at 4°. This suggests that the action of PEP is mediated by an enzymatic mechanism since at temperatures which are closer to the optimum for glycolytic activity a dramatic increment in P_{50} is observed. In our work with species other than human (21) we have observed that the reduction in hemoglobin-oxygen affinity associated with PEP

TABLE IV. EFFECT OF INCUBATION WITH PEP ON 2,3-DPG CONTENT OF RED CELLS STORED IN CPD

		2,3-DPG (μmol/g Hgb)			
		Post-CPD[1,2]		Post-PEP[1,2]	
Day	Initial[1,2]	1 hr	4 hr	1 hr	4 hr
0	10.42 ± 1.13[b]	8.00 ± 1.32[a,b]	3.90 ± 2.38[a]	24.97 ± 4.57[c]	42.85 ± .15[d]
7	10.67 ± 2.17[b]	8.47 ± 3.52[a,b]	5.23 ± 1.99[a]	20.83 ± 3.29[c]	48.13 ± 6.93[d]
14	7.22 ± 0.84[a]	3.50 ± 1.31[a]	2.87 ± 0.94[a]	16.00 ± 3.16[b]	41.70 ± 1.56[c]
21	2.48 ± 1.66[a]	1.67 ± 0.44[a]	0.63 ± 0.38[a]	16.50 ± 0.47[b]	38.30 ± 1.97[c]
28	0.84 ± 0.35[a]	0.50 ± 0.85[a]	0.37 ± 0.12[a]	14.80 ± 0.75[b]	33.10 ± 1.97[c]
35	0.22 ± 0.10[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	12.07 ± 0.20[b]	33.97 ± 1.39[c]
42	0.33 ± 0.20[a]	0.83 ± 0.42[a]	0.10 ± 0.12[a]	11.53 ± 0.54[b]	31.10 ± 3.07[c]

[1] Each value represents mean ± SEM; $n = 3$.
[2] On each sampling day, analysis of variance was performed initially; Duncan's multiple range test was then performed to determine which means varied. Means within rows with same superscripts are not significantly different.

TABLE V. EFFECT INCUBATION WITH PEP ON ATP OF RED CELLS STORED IN CPD

		ATP (μmol/g Hgb)			
		Post-CPD[1,2]		Post-PEP[1,2]	
Day	Initial[1,2]	1 hr	4 hr	1 hr	4 hr
0	4.76 ± 0.25[b]	4.47 ± 0.30[b]	3.53 ± 0.24[a]	5.60 ± 0.49[b]	4.80 ± .33[b]
7	4.25 ± 0.16[c]	3.20 ± 0.44[b]	2.17 ± 0.12[a]	4.23 ± 0.09[c]	3.67 ± 0.64[b,c]
14	3.77 ± 0.38[b,c]	3.00 ± 0.35[b]	1.57 ± 0.18[a]	3.90 ± 0.27[c]	3.80 ± 0.25[b,c]
21	2.70 ± 0.25[b,c]	2.03 ± 0.36[b]	1.23 ± 0.20[a]	3.60 ± 0.30[d]	3.13 ± 0.23[c]
28	2.28 ± 0.19[b]	1.83 ± 0.24[a,b]	1.20 ± 0.15[a]	3.30 ± 0.40[c]	3.17 ± 0.23[c]
35	2.32 ± 0.30[a,b]	1.87 ± 0.18[a]	1.43 ± 0.39[a]	3.73 ± 0.43[c]	3.20 ± 0.36[b,c]
42	1.80 ± 0.12[b]	1.57 ± 0.19[b]	0.97 ± .09[a]	2.87 ± 0.15[c]	2.67 ± 0.23[c]

[1] Each value represents mean ± SEM; $n = 3$.
[2] On each sampling day, analysis of variance was performed initially; Duncan's multiple range test was then performed to determine which means varied. Means within rows with same superscripts are not significantly different.

incubation is limited to those species whose hemoglobin is sensitive to 2,3-DPG, although in some cases, the PEP effect is limited by species specific differences in membrane transport. Furthermore, PEP fails to react directly with stroma-free hemoglobin (P. J. Scannon, unpublished data).

At the pH of incubation required for PEP transport, it is unlikely that 2,3-DPG is generated by the upper portion of the glycolytic pathway since phosphofructokinase, the rate-limiting enzyme, is certainly inhibited. We observed no difference in final glucose concentration following incubation in CPD or CPD plus PEP (data not shown). The mechanism of accumulation of 2,3-DPG observed in red blood cells which have been treated with PEP may be comparable to that observed in pyruvate kinase deficiency where a defect occurring late in glycolytic pathway results in an abnormal accumulation of PEP, 3-phosphoglycerate, and 2,3-DPG (22). In patients who suffer from this hereditary abnormality of red cell glycolysis, 2,3-DPG levels may be two to three times greater than normal and P_{50} may be significantly elevated (23, 24). The coincident maintenance of ATP which was observed in the present studies, however, suggests that pyruvate kinase function persists and that some PEP is dephosphorylated to produce pyruvate. This is confirmed by the attendant increments of pyruvate noted by Tomoda *et al.* (1) in their initial experiments.

PEP is a high-energy intermediate of the glycolytic pathway. As an intermediate compound, it is usually rapidly dephosphorylated in the presence of magnesium and potassium ions to form pyruvate. This reaction is catalyzed by pyruvate kinase and results in production of ATP. This is

TABLE VI. EFFECT OF EXCHANGE TRANSFUSION ON *In Vivo* 2,3-DPG P_{50} IN RATS

	Control[1,2]	PEP-treated[1,2]
Pretransfusion 2,3-DPG (μm/g hgb)	21.25 ± 1.60	22.04 ± 0.87
Pretransfusion P_{50} (Torr)	39.64 ± 1.24	37.41 ± 1.03
Post-transfusion 2,3-DPG (μm/g Hgb)	21.81 ± 1.34	27.58 ± 1.44*
Post-transfusion P_{50} (Torr)	39.28 ± 2.55	46.0 ± 2.37*
Post-transfusion pH	7.39 ± .03	7.37 ± .02

[1] Each value represents mean ± SEM of samples from four rats.
[2] Paired t test was used to compare pre- and post-transfusion 2,3-DPG content and P_{50} of blood from transfused rats. Asterisk (*) indicates a significant difference ($P < 0.05$).

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using optional additive systems containing ascorbate-2-phosphate. Submitted for publication.

15. Weed RT, LaCelle PC. ATP dependence of erythrocyte membrane deformability. In Greenwalt TJ. (Ed.), Red Cell Membrane Structure and Function, Philadelphia, Lippincott, 1969, p. 318.
16. Weed RT, LaCelle PC, Merrill EW. Metabolic dependence of red cell deformability. J Clin Invest 48:795, 1969.
17. LaCelle PC. Alterations of deformability of the erythrocyte in stored blood. Transfusion 9:238, 1969.
18. Nakao K, Wada T, Kamiyama T. A direct relationship between adenosine triphosphate and *in vivo* viability of erythrocytes. Nature (London) 194:877, 1962.
19. Dern RJ, Brewer GJ, Wirokowski JJ. Studies on the preservation of human blood. II. The relationship of erythrocyte adenosine triphosphate levels and other *in vivo* measures to red cell storageability. J Lab Clin Med 69:968, 1967.
20. Sugita Y, Simon ER. The mechanism of action of adenine in red cell preservation. J Clin Invest 44:629, 1965.
21. Scott RL, Sohmer PR. Comparative aspects of the effect of phosphoenolpyruvate on mammalian erythrocyte metabolism. Clin Res 29:42A, 1981.
22. Minakami S, Yoshikawa H. Studies on erythrocyte glycolysis. II. Free energy charges and rate limiting steps in erythrocyte glycolysis. J Biochem 59:139, 1966.
23. Tanaka KR, Paglia DE. Pyruvate kinase deficiency. Semin Hematol 8:367, 1966.
24. Oski FA, Marshall BA, Cohem PJ. Exercise with anemia: The role of the left shifted or right shifted oxygen hemoglobin equilibrium curve. Ann Intern Med 74:44, 1971.
25. Hasart E, Jacobasch G, Rapoport S. Der Einfluss der Temperature auf die Regulation der glycolyse in Erthrozyten des Menchen und der Ratte. Acta Biol Med German 24:725, 1970.

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Effects of Sodium Chloride on Prostacyclin-Stimulated Renin Release in Dogs with Filtering and Nonfiltering Kidneys (41473)

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Abstract. The effects of intrarenal infusion of sodium chloride (NaCl) on prostacyclin (PGI_2)-stimulated hyperreninemia were examined in groups of anesthetized dogs with either a single filtering kidney or a single denervated nonfiltering kidney, a model in which the renal tubules are damaged, and the macula densa is nonfunctional. After control observations, intrarenal infusion of prostacyclin at nonhypotensive doses resulted in significant increments of renin secretion and renal blood flow (RBF) in both preparations. Superimposition of intrarenal NaCl to the ongoing prostacyclin infusion produced a striking decrease of renin secretion in dogs with a filtering kidney. In contrast, dogs with a nonfiltering kidney failed to show a significant change in renin secretion during intrarenal NaCl administration. Renal blood flow remained unaffected by NaCl in both groups. The increment in renal venous plasma sodium concentration of 18–21 meq/liter was similar in both series. It is proposed that the renin response to intrarenal NaCl was mediated through the renal tubules, since renin secretion failed to decrease in the nonfiltering kidney preparation. Thus, the present results indicate that prostacyclin-stimulated renin secretion was modulated by a tubular mechanism, probably the macula densa.

The influence of the renal prostaglandins on renin secretion is well established. It has been demonstrated that intrarenal infusions of arachidonic acid and various prostaglandins, including PGI_2, PGE_2, and PGD_2 can stimulate renin secretion in the dog (1–5). Of the several known renal prostaglandins, it has been suggested that prostacyclin may play a major physiological role in the release of renin (4, 6). Indeed, Frölich *et al.* (7) have postulated that prostaglandin-dependent renin release is probably mediated by PGI_2. Although the mechanism of prostacyclin stimulation of renin is not well defined, studies *in vitro* (8, 9) have demonstrated that PGI_2 exerts a direct action on the juxtaglomerular (JG) cells. Prostacyclin stimulation of renin secretion also has been demonstrated in the nonfiltering as well as the filtering kidney preparation of anesthetized dogs (2, 4, 10). Therefore, it appears that a tubular or macula densa mechanism is not essential for the *in vivo* response to prostacyclin infusion. However, in these earlier studies (4, 10) during intrarenal prostacyclin administration into filtering kidneys, a marked natriuresis also occurred; it is possible that this effect could have attenuated the PGI_2-dependent hyperreninemic response. These considerations, combined with the abundant physiological data which support an important role for a tubular or macula densa mechanism in the control of renin secretion (11, 12), led us to design the present experiments to examine the potential influence of the renal tubular system on prostacyclin-stimulated hyperreninemia. Thus, changes in PGI_2-stimulated renin release were studied in either single filtering or denervated nonfiltering kidneys of dogs before and during the intrarenal infusion of hypertonic NaCl. In a previous investigation, Shade *et al.* (13) demonstrated an inhibition of the hypersecretion of renin secondary to chronic caval constriction during intrarenal infusion of hypertonic NaCl into dogs with filtering kidneys, but not in dogs with nonfiltering kidneys. Therefore, the present experimental design is an approach to examine the importance of the renal tubules and pre-

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sumably the macula densa in the control of prostacyclin-induced hyperreninemia.

Methods. The study involved 13 female mongrel dogs with body weights between 13 and 22 kg. Prior to the acute experiment, the dogs were housed in individual metabolism cages and maintained on a diet that provided approximately 65 meq of sodium and 35 meq of potassium daily for at least 4 days before the acute study and until sodium excretion approximated sodium intake. Water was available *ad libitum*.

All acute experiments were performed in the postabsorptive state. On the morning of the experiment, the dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv), and supplemental small doses of the drug were administered as needed to maintain a satisfactory level of anesthesia. Polyethylene catheters (fr. 8) were inserted into the femoral vessels and their tips advanced to the inferior vena cava and abdominal aorta. The arterial catheter was connected via a Statham P23Db strain gauge pressure transducer to a Hewlett Packard 7702B recorder in order to monitor mean arterial pressure (MAP). The femoral vein catheter was used for the administration of creatinine and replacement of blood. Both kidneys were exposed via retroperitoneal flank incisions and a unilateral nephrectomy was performed to remove any influence of this kidney on renin secretion. The contralateral renal artery was fitted with an electromagnetic flow probe connected to a model 501 Carolina Electronics flowmeter for determination of renal blood flow. Number 22-gauge needles attached to polyethylene tubes were inserted in the renal artery (distal to the flow probe) for infusion of solutions with a Harvard syringe pump and into the renal vein in order to obtain renal venous blood samples. A ureteral polyethylene 100 catheter was also positioned near the renal pelvis and urine was collected in a graduated cylinder. After a priming solution of creatinine was given, a constant infusion was maintained throughout the experiment in order to determine creatinine clearance (C_{Cr}). In addition, an isotonic NaCl infusion into the renal artery was begun. All intrarenal solutions including prostacyclin, isotonic NaCl, and hypertonic NaCl were given at a constant rate of 0.59 ml/min. Prostacyclin sodium salt was prepared by dissolving 1 mg in 1 ml of 1 *M* Tris buffer; this solution was diluted to 100 ml with 0.05 *M* Tris buffer (pH 9.4). The final dilution for the desired concentration was made with isotonic NaCl. Concentrations of plasma arterial sodium, potassium, and creatinine were determined from blood samples obtained at the midpoint of each period and in the urine collected over the entire clearance interval. Blood for the measurements of renal venous plasma sodium (RVP Na) was obtained at the end of each period. From these data, C_{Cr} and electrolyte excretion were calculated. During the last 4 min of each period, renal venous and arterial blood samples were collected for measurement of plasma renin activity (PRA) and hematocrit. Mean arterial pressure and RBF were recorded continuously. Following surgical preparation, a 60-min equilibration period was allowed before initiation of the following two experimental series. In all experiments, blood withdrawn for sampling was replaced with an equal volume of fresh donor blood from a normal dog.

Experiment 1. Intrarenal PGI_2 and superimposed NaCl infusion in anesthetized dogs with a single filtering kidney ($N = 7$). Three 15-min control renal clearance periods with intrarenal infusion of isotonic NaCl were obtained. In the subsequent two periods, prostacyclin was given intrarenally at a rate of 1.0×10^{-8} g/kg/min. This was followed by two periods of prostacyclin infusion at a rate of 1.5×10^{-8} g/kg/min. This latter dose of prostacyclin was continued throughout the remainder of the experiment in order to achieve maximal stimulation of renin release without a concomitant drop in MAP. During the next three periods, PGI_2 and hypertonic NaCl were given together intrarenally. The hypertonic NaCl solution was administered in a concentration calculated to increase the existing RVP Na concentration an additional 20 meq/liter for each individual experiment and the range for the group was 2.42–5.84 meq/min. Finally, two recovery

periods with infusion of PGI_2 alone were obtained.

Experiment 2. Intrarenal PGI_2 and superimposed NaCl infusion in anesthetized dogs with a single denervated nonfiltering kidney ($N = 6$). A denervated nonfiltering kidney was produced in each dog 4 days before the acute experiment. The nonfiltering kidneys were prepared according to the method of Blaine *et al.* (14). Briefly, under anesthesia with pentobarbital, one of the dog's kidneys was exposed via a retroperitoneal flank incision. The renal artery was occluded for 2 hr with a serrafine clamp. After restoration of blood flow, the ureter was ligated and cut. Finally, the kidney was denervated. Four days later the acute experiment was performed and an identical protocol described for series No. 1 was followed, except that due to the absence of filtering capacity, ureteral catheterization was omitted; consequently, measurements of C_{Cr} and electrolyte excretion were not obtained. Also in this series, the concentration of hypertonic NaCl infusion was adjusted to increase the existing RVP Na concentration an additional 20 meq/liter for each individual experiment. Since the renal plasma flow in the nonfiltering kidney was substantially reduced when compared to the filtering kidney, the rate of infusion of hypertonic NaCl was less than in the filtering kidney series, and it ranged from 1.6 to 2.5 meq/min. At the end of each experiment, these kidneys were decapsulated and a solution of lissamine green dye was injected into the renal artery to verify the nonfiltering status. This was accomplished by the failure to observe the appearance of dye within the renal tubules. This criterion was fulfilled in each of the dogs reported in this series.

Analytical methods. Plasma and urine electrolyte and creatinine concentrations were determined by standard techniques. PRA was measured by radioimmunoassay for angiotensin I(A-I) with the technique described by Sealey et al., (15). Renin secretion rate was calculated by multiplying the difference between renal venous and arterial PRA concentration by renal plasma flow. Renal plasma flow (RPF) was calculated from the formula RPF = RBF × 1 − HCT. Hematocrits were determined by the microcapillary tube method.

The results are presented as an average of the control and each of the different experimental categories. The data were analyzed by the χ^2 sign test for differences between categories. A P value of <0.05 was considered significant.

Results. *Experiment 1. Effects of intrarenal PGI_2 and hypertonic NaCl infusion in anesthetized dogs with a single filtering kidney.* Infusions of PGI_2 at nonhypotensive doses of 1.0 and 1.5 × 10^{-8} g/kg/min significantly increased renin secretion from 147 ± 70 to 357 ± 146 and 416 ± 131 ng A-I/min, respectively (Fig. 1). Superimposition of hypertonic NaCl during PGI_2 administration resulted in an increase in RVP Na concentration of approximately 18 meq/liter ($P < 0.05$) (Table I) and a threefold increase in renal sodium excretion ($P < 0.05$) (Table I). Also, the filtered load of sodium appeared to increase in 6 out of 7 animals although the increment did not reach the 5% level of significance for the group. These changes were correlated with a significant reduction of renin secretion from 416 ± 131 to 157± 51 ng A-I/min (Fig. 1), a value that was not different from con-

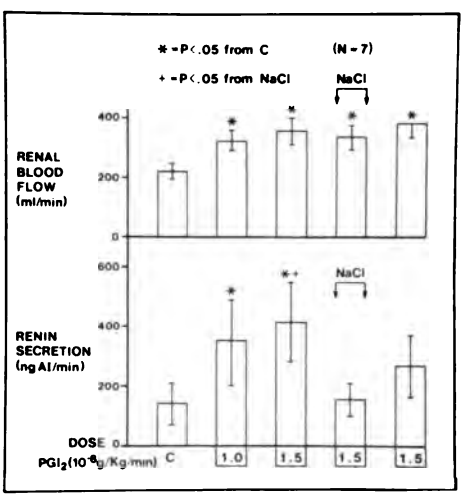

FIG. 1. Responses to intrarenal PGI_2 and NaCl infusions in dogs with a single filtering kidney. Values are expressed as means ± SE.

TABLE I. CHANGES IN CARDIOVASCULAR AND RENAL HEMODYNAMIC FUNCTION DURING PGI_2 AND NaCl INFUSION INTO FILTERING KIDNEYS OF ANESTHETIZED DOGS ($N = 7$)

		PGI_2 infusion at 1.0 or .5 × 10^{-8} g/kg/min				
				NaCl		
	C	1.0	1.5	1.5	1.5	
MAP mm Hg	134 ± 4	133 ± 5	131 ± 4	131 ± 5	131 ± 4	
RVR mm Hg/[ml/min]	0.67 ± 0.10	0.44* ± 0.06	0.40* ± 0.06	0.42* ± 0.05	0.38* ± 0.05	
C_{Cr} ml/min	39 ± 3	40 ± 5	42 ± 5	38 ± 4	39 ± 5	
RVP Na meq/L	143 ± 1	143 ± 1	143 ± 1	161*,† ± 2	154 ± 3	
RVP K meq/L	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.1*,† ± 0.1	3.4* ± 0.1	
E_{Na} μEq/min	76 ± 24	156* ± 30	165* ± 27	538*,† ± 69	520* ± 70	
E_K μEq/min	37 ± 5	58* ± 8	66* ± 8	61 ± 7	90* ± 14	
UV ml/min	0.51 ± 0.15	1.12* ± 0.20	1.25* ± 0.02	2.97*,† ± 0.35	3.01* ± 0.31	

Note. Values are expressed as mean ± SE. Abbreviations are explained in text. Sodium and potassium excretion rates are represented by E_{Na} and E_K. The asterisk (*) indicates statistically significant P value for the noted period compared with control. The dagger (†) indicates statistically significant P value for noted period compared to the immediately preceding one.

trol. During the recovery period with infusion of PGI_2 alone, renin secretion appeared to rise toward the prehypertonic NaCl level, but the increment was not significantly different from the control (Fig. 1); however, both RVP Na and urinary sodium excretion remained elevated during the recovery (Table I). PGI_2 infusion induced a significant increase in the renal blood flow which was not attenuated during hypertonic NaCl administration (Fig. 1). Likewise, renal vascular resistance (RVR) was decreased with PGI_2 infusion (both doses) ($P < 0.05$) and remained attenuated during the hypertonic NaCl infusion and recovery period ($P < 0.05$) (Table I). Creatinine clearance and MAP were not detectably changed throughout the experiment (Table I). PGI_2 significantly increased renal potassium excretion, but there were no further consistent changes in the excretion of this electrolyte during hypertonic NaCl administration (Table I); during the recovery period, potassium excretion was again elevated ($P < 0.05$) (Table I). In addition, renal venous plasma potassium (RVP K) concentration decreased slightly ($P < 0.05$) during hypertonic NaCl infusion and during the recovery period ($P < 0.05$) (Table I).

Experiment 2. Response to intrarenal PGI_2 and hypertonic NaCl infusion in anesthetized dogs with a single denervated nonfiltering kidney. As in experiment 1, infusion of nonhypotensive doses of PGI_2 at a rate of 1.0 and 1.5 × 10^{-8} g/kg/min resulted in a significant increase in renin secretion from 119 ± 30 to 456 ± 53 and 463 ± 42 ng A-I/min, respectively (Fig. 2). Intrarenal infusion of hypertonic NaCl produced a significant increase in RVP Na concentration of approximately 20 meq/liter (Table II). In contrast to series 1, the superimposition of hypertonic NaCl did not suppress renin secretion which remained elevated from the control at a rate of 365 ± 29 ng A-I/min ($P < 0.05$) (Fig. 2). This value was not significantly different from the level of 463 ± 42 ng A-I/min obtained with the infusion of PGI_2 alone (Fig. 2). During the recovery period, renin secretion appeared to remain elevated but the change did not reach statistical significance at the 5%

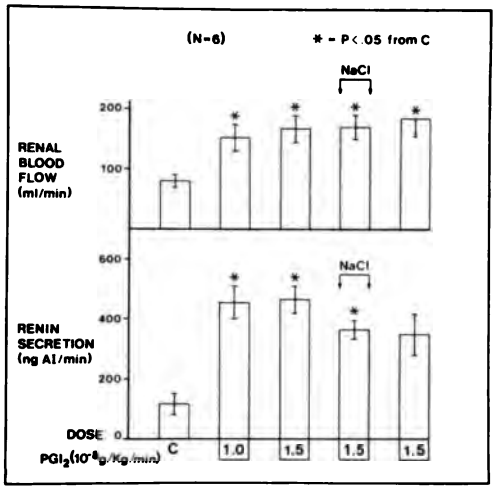

FIG. 2. Responses to intrarenal PGI_2 and NaCl infusions in dogs with a single denervated nonfiltering kidney. Values are expressed as means ± SE.

level. Renal blood flow significantly increased ($P < 0.05$) and RVR decreased ($P < 0.05$) during the administration of PGI_2 and these changes were unaffected by the administration of hypertonic NaCl (Fig. 2, Table II). As with the filtering kidney, RVP K concentration appeared to decrease slightly with the infusion of hypertonic NaCl (Table II), but the change was not significant. Finally, as in the first series, MAP remained unchanged throughout the experiment.

Discussion. The results of the present study demonstrate the active participation of an intact renal tubular mechanism in the modulation of prostacyclin-stimulated renin release. In the present experiments and in agreement with previous reports from this laboratory (4), intrarenal infusion of nonhypotensive doses of PGI_2 significantly stimulated renin release in dogs with either single filtering or nonfiltering kidneys. During this hyperreninemic state, the influence of the renal tubular mechanism was evaluated by intrarenal infusion of hypertonic NaCl, an intervention that is well known to inhibit renin release (13, 16). Accordingly, in our first series of experiments with filtering kidneys, the superimposition of hypertonic NaCl on the ongoing PGI_2 infusion significantly reduced renin secretion to the control level. This suppression was associated with an increment in RVP Na concentration, in the filtered load of sodium, and in sodium excretion. On the other hand, in dogs with a nonfiltering kidney, in an identical protocol NaCl infusion failed to attenuate significantly renin release, even though the increment in RVP Na concentration was similar to the level achieved in dogs with filtering kidneys. Comparison of renin secretion during hypertonic NaCl infusion in the filtering and nonfiltering kidney series clearly indicates that in the absence of a tubular mechanism, hypertonic NaCl was ineffective in reducing renin release. It seems reasonable to suggest that the renal tubular mechanism was mediated by the macula densa in the series with the filtering kidney.

In the present study the nonfiltering kid-

TABLE II. CHANGES IN CARDIOVASCULAR AND RENAL HEMODYNAMIC FUNCTION DURING PGI_2 AND NaCl INFUSION INTO NONFILTERING KIDNEYS OF ANESTHETIZED DOGS ($N = 6$)

		PGI_2 infusion at 1.0 or 1.5 × 10^{-8} g/kg/min			
				NaCl	
	C	1.0	1.5	1.5	1.5
MAP mm Hg	140 ± 4	140 ± 2	140 ± 2	140 ± 2	141 ± 2
RVR mm Hg/ [ml/min]	1.90 ± 0.28	1.02* ± 0.15	0.91* ± 0.12	0.91* ± 0.13	0.86* ± 0.14
RVP Na meq/L	145 ± 1	144 ± 1	143 ± 1	164*,† ± 2	152* ± 1
RVP K meq/L	4.3 ± 0.1	4.4 ± 0.1	4.4 ± 0.2	4.1 ± 0.2	4.4 ± 0.2

Note. Values are expressed as mean ± SE. Abbreviations are explained in the text. The asterisk (*) indicates statistically significant P value for the noted period compared with control. The dagger (†) indicates statistically significant P value for the noted period compared to the immediately preceding one.

neys were denervated whereas the filtering kidneys were innervated. In the filtering kidneys, it seems unlikely that intrarenal NaCl infusion influenced renal nerve activity with a subsequent effect on renin release. In an earlier study by Shade *et al.* (13) in which the renal nerves were intact in both filtering and nonfiltering kidneys, a similar intrarenal infusion of hypertonic NaCl decreased renin secretion only in the filtering kidney.

The renin secretion response during the recovery phase in the filtering and nonfiltering kidney preparations was variable and requires additional comment. In both groups, renin secretion appeared to be elevated above control levels, but the increments were not statistically significant at the 5% level for either group. Similarly, renin secretion in the recovery period was not different from PGI_2 induced renin secretion prior to hypertonic NaCl infusion. It is possible that the observed increased variability in renin secretion during the recovery phase was related to nonspecific secondary mechanisms, possibly volume expansion. Also, in the filtering kidney series RVP Na and sodium excretion remained elevated during the recovery period and this could have continued to attenuate partially the renin response to prostacyclin infusion. These findings were not unexpected, since similar responses in the recovery phase were observed by Shade *et al.* (13) using a similar protocol of hypertonic NaCl infusion in filtering and nonfiltering kidneys of anesthetized dogs with hyperreninemia due to caval constriction.

The bulk of the early evidence in the literature (11, 12, 16) supports the view that an increased tubular sodium concentration or the load of sodium at the macula densa can attenuate renin secretion. More recently, studies by Kotchen *et al.* (17, 18) have postulated that chloride rather than sodium might be the important ion for this effect. In addition, there is the suggestion (11) that both sodium and chloride are involved in the macula densa sensor mechanism. Regardless of which ion(s) are detected by the macula densa, comparative studies with hypertonic NaCl infusion in the filtering and nonfiltering kidney (13) have provided evidence to support an important role for the tubular system in the control of renin secretion. Inhibition of renin release by hypertonic NaCl has been demonstrated in normal anesthetized dogs with baseline renin secretion (16) as well as in dogs with hyperreninemia induced by caval constriction (13) or by norepinephrine (16). These observations and the present data are consistent with the idea that the macula densa can attenuate both basal and stimulated levels of renin secretion. The above considerations are particularly interesting when applied to prostaglandin-dependent renin release. Under appropriate conditions, it is possible that the natriuresis induced by various endogenous prostaglandins could reflect increased NaCl delivery to the macula densa and a feedback mechanism which functions as a brake on the increase in renin release. A similar postulate has been suggested in a recent review by Henrich (19). Studies by Higashihara *et al.* (20) and Stokes (21) have suggested a PGE_2-dependent inhibition of chloride reabsorption in the thick ascending limb of Henle, but the interpretation of the data has been questioned (22) and conclusive evidence of the action of prostaglandins on tubular sodium and chloride transport is lacking.

The effects of prostacyclin on renal hemodynamic function and electrolyte excretion have been well documented and the present results are in agreement with previous studies (4, 10). In both the filtering and nonfiltering kidney series, the elevation in RBF produced by PGI_2 infusion was not altered by the administration of hypertonic NaCl, while renin secretion was strikingly reduced in the filtering kidney preparation. This finding demonstrates that hypertonic NaCl did not inactivate or directly block the biological action of PGI_2. Other investigators have also reported lack of changes in renal hemodynamic function during intrarenal NaCl infusion with similar protocols in sodium replete dogs (16) or in dogs with caval constriction (13).

In summary, hypertonic NaCl inhibited PGI_2-stimulated renin secretion in the

single filtering kidney while hypertonic NaCl had no effect on PGI_2-dependent renin release in the nonfiltering kidney. Thus an intact tubular mechanism is essential for this response. It is suggested that changes in tubular delivery of NaCl to the macula densa can modulate the hyperreninemia induced by PGI_2.

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1. Bolger PM, Eisner GM, Romwell PW, Slotkoff LM. Effect of prostaglandin synthesis on renal function and renin in the dog. Nature (London) 259:244–245, 1976.
2. Gerber JG, Branch RA, Nies AS, Gerkens JF, Shand DG, Hollifield J, Oates JA. Prostaglandins and renin release. II. Assessment of renin secretion following infusion of PGI_2, E_2 and D_2 into the renal artery of anesthetized dogs. Prostaglandins 15:81–88, 1978.
3. Seymour AA, Zehr JE. Influence of renal prostaglandin synthesis on renin control mechanisms in the dog. Circ Res 45:13–25, 1979.
4. Seymour AA, Davis JO, Freeman RH, DeForrest JM, Rowe BP, Williams GM. Renin release from filtering and nonfiltering kidneys stimulated by PGI_2 and PGD_2. Amer J Physiol 237(4):F285–F290, 1979.
5. Yun J, Kelly G, Bartter FC, Smith H, Jr. Role of prostaglandins in the control of renin secretion in the dog. Circ Res 40:459–464, 1977.
6. Oates JA, Whorton AR, Gerkens JG, Branch RA, Hollifield JW, Frölich JC. The participation of prostaglandins in the control of renin release. Fed Proc 38:72–74, 1979.
7. Frölich JC. Prostaglandins: Role in renin regulation and mediation of antihypertensive drug effects. Arch Int Pharmacodyn Ther Suppl:213–224, 1980.
8. Suzuki S, Franco Saenz R, Tan SY, Mulrow PW. Direct action of prostaglandins on renin release from rat renal cortical slices. Proc Soc Exp Biol Med 166:484–488, 1981.
9. Whorton AR, Misorpo K, Hollifield J, Frölich JC, Inagami T, Oates JA. Prostaglandins and renin release. I. Stimulation of renin release from rabbit renal cortical slices by PGI_2. Prostaglandins 14:1095–1104, 1977.
10. Bolger PM, Eisner GM, Romwell PW, Slotkoff LM. Renal actions of prostacyclin. Nature (London) 271:467–469, 1978.
11. Davis JO, Freeman RH. Mechanisms regulating renin release. Physiol Rev 56:1–56, 1976.
12. Freeman RH, Davis JO, Gotshall RW, Johnson JA, Spielman WS. The signal perceived by the macula densa during changes in renin release. Circ Res 35:307–315, 1974.
13. Shade RE, Davis JO, Johnson JA, Witty RT. Effects of renal arterial infusion of sodium and potassium on renin secretion in the dog. Circ Res 31:719–727, 1972.
14. Blaine EG, Davis JO, Witty RT. Renin release after hemorrhage and after suprarenal aortic constriction in dogs without sodium delivery to the macula densa. Circ Res 28:1081–1089, 1979.
15. Sealey JE, Laragh JH, Gerten-Barnes J, Accto RM. The measurement of plasma renin activity in man. In: Hypertension Manual. Yorke Medical Books/Dunn–Donnelley, New York, pp621–640, 1974.
16. Nash FD, Rostorfer HH, Bailie MD, Wathen RL, Schneider EG. Renin release in relation to renal sodium load and dissociation from hemodynamic changes. Circ Res 22:473–487, 1968.
17. Kotchen TA, Galla JH, Luke RG. Contribution of chloride to the inhibition of plasma renin by sodium chloride in the rat. Kidney Int 13:201–207, 1978.
18. Kotchen TA, Krzyzaniak KE, Anderson JE, Ernst CB, Galla JH, Luke RG. Inhibition of renin secretion by HCl is related to chloride in both dog and rat. Amer J Physiol 239(8):F44–F49, 1980.
19. Henrich WL. Role of prostaglandins in renin secretion. Kidney Int 19:822–830, 1981.
20. Higashihara E, Stokes JG, Kokko JP, Campbell WB, DuBose TD, Jr. Cortical and papillary micropuncture examination of chloride transport in segments of the rat kidney during inhibition of prostaglandin production. J Clin Invest 64:1277–1287, 1979.
21. Stokes JB. Effect of prostaglandin E_2 on chloride transport across the rabbit thick ascending limb of Henle. J Clin Invest 64:495–502, 1979.
22. Fine LG, Kirschenbaum MA. Absence of direct effects of prostaglandins on sodium chloride transport in the mammalian nephron. Kidney Int 19:797–801, 1981.

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Differences in the Reported Frequencies of Cleft Lip Plus Cleft Lip and Palate in Asians Born in Hawaii and the Continental United States[1] (41474)

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Abstract. The etiology of cleft lip with or without cleft palate (CL/CP) is not known but present evidence suggests that both genetic and environmental factors act to determine susceptibility. Race has been reported to exert a strong influence on the incidence of CL/CP; e.g., the frequency of CL/CP is nearly twice as high among Japanese born in Japan or Hawaii as it is among Caucasians in Hawaii, Western Europe, and North America, and the risk in American blacks is one-half that in Caucasians. This notwithstanding, a survey of Los Angeles Hospitals and Clinics for families with facial clefting revealed no Orientals despite the fact that this group constitutes 6% of the population. To investigate the possibility that the rate of clefting had declined in Orientals' data was obtained from birth certificates from Hawaii, California, and New York, and from the USPHS Birth Defects Monitoring Program. The results suggest that the frequency of CL/CP but not isolated CP may be significantly lower among Japanese and other Orientals born in California and New York than among those born in Japan or Hawaii. This implies that environmental factors play a major role in determining the frequency of CL/CP in this racial group in the Orient and Hawaii.

Isolated cleft palate (CP) and cleft lip with or without cleft palate (CL/CP) are congenital malformations of unknown etiology. Both anomalies tend to recur in families and also to vary in incidence with season and year of conception, and parental social class, age, parity, ethnic origin, and place of residence during pregnancy (1–4). It is not clear whether the variations in the frequency of CP and CL/CP observed among populations and races are predominantly genetic or environmental in origin, because in past studies most of the groups compared differed in both respects.

The influence of race on the incidence of CP and CL/CP appears to be especially strong (5). The frequency of cleft lip with or without cleft palate has been reported to be nearly twice as high among Japanese born in Japan or Hawaii as it is among Caucasians born in Hawaii, Europe, or North America (6, 7) and the frequency of CP in blacks is approximately one-half that in American Caucasians (1). This notwithstanding, a survey of Los Angeles Hospitals and Clinics for families with facial clefting revealed no Orientals even though this group constitutes 6% of the population. It was concluded that either Oriental patients were going to private physicians not affiliated with mainstream medical institutions or the incidence of clefting was low.

In an effort to determine if the frequency of facial clefting in the Oriental population in California had truly declined, data from birth certificates were obtained from the States of Hawaii, California, and New York, and hospital discharge diagnoses were secured from the USPHS Birth Defects Monitoring Program. The results suggest that the frequency of CL/CP but not CP may be significantly lower among Japanese and other Orientals born in California and New York than among those born in Japan or Hawaii. This implies that environmental factors play a major role in

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determining the frequency of CL/CP in this racial group in the Orient and Hawaii.

Methods. Computer printouts giving total live births, and the number of isolated cleft palates, cleft lips, and cleft lips plus cleft palates with the race of the mother and father were obtained from the Departments of Health of the States of Hawaii, California, and New York. In addition, data were obtained from the Birth Defects Monitoring Program, USPHS Center for Disease Control, Atlanta, Georgia. The data from the USPHS CDC do not contain information on the race of mother or father, and all Orientals are recorded as "Asians." Approximately 20% of all "Asian" births were reported from the State of Hawaii.

Isolated cleft palate and cleft lip with or without cleft palate are considered to have distinct genetic and environmental etiologies (1, 7). Because ascertainment varies between studies, comparisons of frequencies of CP and CL/CP were made between races within each study only. It is assumed that nonascertainment within a study will be equally distributed over racial subgroups of the population as was the case in Hawaii as reported by Ching and Chung (7). With the exception of the data from the Birth Defects Monitoring Program the information reported is derived from incrosses only.

Statistical analyses were done by Leslie Bernstein, Ph.D. The Mantel–Haenszel test (8) was used to test the hypothesis of equal incidence (risk ratio = 1) among Caucasians and Japanese or Asians.

Results. *Isolated cleft palate among Caucasians and Japanese born in Hawaii or California (Table I).* The frequencies of isolated cleft palate among Japanese in Hawaii and California were consistently lower than those found among Caucasians; however, the differences were not statistically significant.

Cleft lip and cleft lip plus cleft palate among Caucasians and Japanese born in Hawaii or California (Table I). The relative risk of Japanese in Hawaii producing progeny with CL/CP was 1.85 to 2.41 times higher than that in Caucasians; however, in

TABLE I. ISOLATED CLEFT PALATE (CP) AND CLEFT LIP PLUS CLEFT PALATE (CL/CP) AMONG CAUCASIANS AND JAPANESE BORN IN HAWAII OR CALIFORNIA

	Caucasian		Japanese		CP	Caucasian	Japanese	CL/CP
	Number of live births	CP per 1000 births	Number of live births	CP per 1000 births	Relative risk	CL/CP per 1000 births	CL/CP per 1000 births	Relative risk
Hawaii								
1948–1966[a]	77,013	0.43	67,608	0.65	1.51	0.98	1.82	1.85*
1974–1976[b]	11,914	0.67	4,650	0.00	—	0.58	1.50	2.41*
California								
1968–1973[c]	2,807,851	0.27	28,238	0.18	0.63	0.58	0.46	0.79
1974–1977[c]	1,081,126	0.33	5,463	0.00	—	0.67	0.55	0.82

[a] Ref. (7).
[b] Data from birth certificates supplied by the Department of Health, State of Hawaii.
[c] Data from birth certificates supplied by the Department of Health, State of California.
* $P < 0.05$.

California during roughly the same period the risk was essentially the same.

Isolated cleft palate among Caucasians and Asians born in Hawaii or in the United States (Table II). The relative risk of Chinese, Japanese, or Filipinos, either as individual races where those data were available or as "Asians," producing progeny with CP in Hawaii, California, New York or in other areas of the United States was consistently but not significantly below that of Caucasians.

Cleft lip and cleft lip plus cleft palate among Caucasians and Asians born in Hawaii or in the United States (Table II). The relative risk of CL/CP was higher in all Asian racial groups in Hawaii (1.53 to 2.41, individual data not shown), but in California and New York the frequencies were somewhat lower than that of Caucasians. The relative risk of CL/CP in Asians (1.20) from the USPHS data (1970–1979) was not significantly higher than that of Caucasians even though approximately 20% of the Asian births were reported from Hawaii. (From this one would reason that the risk among Asians born in the continental USA is lower than 1.20.)

Discussion. Opinions differ on whether the variations in the frequencies of facial clefting between racial groups are due primarily to differences in the environment (9) or in the population frequency of particular combinations of genes which influence development (5). The findings presented here support the view that the variations in frequencies of CL/CP noted between Caucasians and Japanese and other Orientals born in Hawaii and the United States are secondary to environmental changes.

The few studies which have reported differences in the frequency of clefts among races agree that clefts are seen much less frequently among blacks than among Caucasians from the same geographic area. It has been reported that only seven cleft cases were observed among 12,520 black births (0.55/1000) at Johns Hopkins Hospital. During the same period there were 17 cases among 15,656 Caucasian births (1.06/1000) (10). Other studies based on

TABLE II. ISOLATED CLEFT PALATE AND CLEFT LIP AND CLEFT LIP PLUS CLEFT PALATE AMONG CAUCASIANS AND ASIANS BORN IN HAWAII OR IN THE UNITED STATES

	Caucasian		Asian[a]			CP		Caucasian	Asian	CL/CP	
	Number of live births	CP per 1000	Number of live births	CP per 1000	Relative risk	χ^2	CL/CP per 1000 births	CL/CP per 1000 births	Relative risk	χ^2
Hawaii, 1974–1976	11,914	0.67	10,858	0.09	0.13	3.50	0.58	1.19	2.05	3.14
California, 1974–1977	1,081,126	0.33	29,844	0.20	0.60	1.92	0.66	0.50	0.76	1.00
USPHS,[b] 1970–1979	7,334,169	0.54	58,822	0.57	1.05	1.22	1.01	1.22	1.20	2.21
New York State,[c] 975 1979	843,190	0.21	11,355	0.26	1.25	0.03	0.53	0.35	0.66	0.63

[a] Japanese, Chinese, and Filipino.
[b] Data from the USPHS Birth Defects Monitoring Program.
[c] Data from birth certificates supplied by the Department of Health, State of New York.

birth certificate data have reported consistently lower attack rates of facial clefting among American blacks than among Caucasians (11–13) and this relationship is maintained despite geographic and cultural change (2). Although these studies strongly suggest that genetic differences primarily account for the variations in attack rates for CP and CL/CP between Blacks and Caucasians, as with all studies of this type the differences may be caused by unrecognized variations in ascertainment, local environment, racial classification, or diagnostic criteria. Some of the problems associated with the use of birth certificates in the epidemiologic assessment of facial clefts are addressed in Refs. 16 and 17.

Ching and Chung (7) reported that the Caucasian incidence of oral clefts in Hawaii was approximately the median value found among other Caucasoid populations studied (14) and that the incidence of CL/CP for Japanese was significantly higher and intermediate between the incidences estimated by Neel (5), Koguchi (6), and Kobayashi (15) for Japanese in Japan suggesting that the racial frequencies were not altered significantly by the migration of the Japanese and Caucasians to Hawaii (the frequency of CP among Japanese born in Hawaii was slightly higher than that estimated for Japanese in Japan). The data presented here support those findings but suggest strongly that when the Japanese and perhaps other Asians migrate to California, New York, or other areas of mainland United States the frequency of CL/CP but not CP decreases to approximate that noted in Caucasians.

There are three explanations for the differences observed in the frequency of CL/CP among the Japanese living in Hawaii or California and among other Asians living in Hawaii and the United States. There may be cultural differences in the reporting frequency (16, 17), migration to the mainland may not be representative of the entire population or there may have been a true decrease in the occurrence of this anomaly in these racial groups as a function of time or assimilation into a different culture and environment. At present there is no evidence in support of the first two possibilities but observations exist which support the concept that attack rates for facial clefting in certain racial groups do vary with geography and environment (1, 2, 4). Prominent among changes which occur with migration is diet. Oriental and Hawaiian diets tend to be lower in fat and animal protein and higher in fish, vegetables and vitamin A than does the average western diet (18, 19). This may be of specific interest because it is known that high dietary levels of vitamin A increase susceptibility to cleft palate in mice bearing H-2D^b alleles in their major histocompatibility complex (20).

Finally, the observation that the frequency of isolated cleft palate among the Japanese did not vary with time and place as did that of CL/CP tends to support the view that these anomalies have distinct etiologies.

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1. Greene JC. Epidemiology of congenital clefts of the lip and palate. Public Health Reports, Public Health Service, U.S. Department of Health, Education and Welfare, Vol 78:pp589–602, 1963.
2. Leck I. Ethnic differences in the incidence of malformations following migration. Brit J Prev Soc Med 23:166–173, 1969.
3. Wehrung DA, Hay S. A study of seasonal incidence of congenital malformations in the United States. Brit J Prev Soc Med 24:24–32, 1970.
4. Saxen I. Epidemiology of cleft lip and palate. Brit J Prev Soc Med 29:103–110, 1975.
5. Neel JV. A study of major congenital defects in Japanese infants. Amer J Hum Genet 10:398–445, 1958.
6. Koguchi H. Population data on cleft lip and cleft palate in the Japanese. In Melnick M, Bixler D, Shields ED, eds. Etiology of Cleft Lip and Cleft Palate. New York, Liss, pp297–323, 1980.
7. Ching GSH, Chung CS. A genetic study of cleft lip and palate in Hawaii. Amer J Hum Genet 26:162–176, 1974.

8. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Nat Cancer Inst 22:719–748, 1959.
9. Morton NE. Genetics of inter-racial crosses in Hawaii. Eugen Q 9:23–29, 1962.
10. Davis JS. The incidence of congenital clefts of the lip and palate. Ann Surg 80:363–374, 1924.
11. Loretz W, Westmoreland WW, Richards LF. A study of cleft lip and cleft palate births in California, 1955. Amer J Public Health 51:873–877, 1961.
12. Grace LG. Frequency of occurrence of cleft palates and harelips. J Dent Res 22:495–497, 1943.
13. Lutz KR, Moor FB. A study of factors in the occurrence of cleft palate. J Speech Hearing Dis 20:271–276, 1955.
14. Leck I. The etiology of human malformations: Insights from epidemiology. Teratology 5:303–314, 1972.
15. Kobayoshi Y. A genetic study on harelip and cleft palate. Jap J Hum Genet 3:73–107, 1958.
16. Mackeprang M, Hay S, Lunde AS. Completeness and accuracy of reporting of malformations on birth certificates. Health Services and Mental Health Administration Health Reports 87:43–49, 1972.
17. Meskin LH, Pruzansky S. Validity of the birth certificate in the epidemiologic assessment of facial clefts. J Dent Res 46:1456–1459, 1967.
18. Hankin JH, Nomura A, Rhoads GG. Dietary patterns among men of Japanese ancestry in Hawaii. Cancer Res 35:3259–3264, 1975.
19. Kagan A, et al. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California; demographic, physical, dietary and biochemical characteristics. J Chron Dis 27:345–364, 1974.
20. Tyan ML, Miller KK. Genetic and environmental factors in cortisone-induced cleft palate. Proc Soc Exp Biol Med 158:618–621, 1978.

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Ovine Maternal and Fetal Circulatory Responses to an Endoperoxide Analog (41475)

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Abstract. There are extensive data on the circulatory responses to prostaglandins during pregnancy, but little is known about the precursor endoperoxides. The endoperoxide analog (15S)-hydroxy-9α,11α-(epoxymethano)prosta-5Z,13E-dienoic acid (EPA_1) was used to evaluate the circulatory effects of endoperoxides in pregnant sheep. Ten near-term ewes and their fetuses were chronically catheterized to permit the measurement of regional blood flows by the radioactive microsphere method. In five sheep a fetal IV bolus injection of 12.5 μg/kg EPA_1 produced a significant increase in fetal blood pressure from 43 to 51 mm Hg, vascular resistance of the cotyledons from 0.06 to 0.09 $PRU \cdot kg^{-1}$, membranes from 0.84 to 3.15 $PRU \cdot kg^{-1}$, and kidneys from 1.18 to 1.94 $PRU \cdot kg^{-1}$. In five sheep a 10-min maternal infusion of 0.5 μg/kg/min EPA_1 produced a significant increase in blood pressure from 89.0 to 115.0 mm Hg, resistance of the uterus from 0.49 to 1.10 PRU, and kidneys from 0.13 to 0.20 PRU. There was no significant change in maternal cotyledonary resistance. It is evident from these results that EPA_1 causes widespread vasoconstriction. In the placenta, however, there is vasoconstriction on the fetal side but the resistance of the maternal vascular bed is unaltered.

The endoperoxides are intermediaries in the formation of the prostaglandins, are biologically active, and exert strong and independent pharmacologic and physiologic effects on vascular and respiratory smooth muscle (1). For several years we have investigated the role of prostaglandins in regulating and modulating placental blood flows (2–6). The actions of the endoperoxides are not well documented. The half-life of the endoperoxides is 4 to 5 min (7, 8) and some of the circulatory effects attributed to the prostaglandins may well be due to endoperoxide action (1). The endoperoxides derived from arachidonic acid are prostaglandins G_2 (PGG_2) and H_2 (PGH_2). These are powerful vasoconstrictors, bronchoconstrictors, and platelet aggregating agents (7). As there is relatively little information on maternal and fetal cotyledonary responses to endoperoxides, this study was designed to evaluate these responses in pregnant sheep. The substance used in the experiments was the stable endoperoxide analog (15S)-hydroxy-9α,11α-(epoxymethano)prosta-5Z,13E-dienoic acid (EPA_1) (9).

Materials and Methods. Ten cross-bred sheep were used in this study. Surgical preparation was performed between Day 125 and Day 128 of gestation. Anesthesia was induced with an intramuscular injection of ketamine (10 mg/kg) and atropine (0.6 mg). Anesthesia was maintained with a continuous intravenous infusion of ketamine in normal saline (10 mg/min). A maternal left ventricular catheter was placed via the right carotid artery with the use of local xylocaine to supplement the parenteral anesthesia. A polyethylene catheter (PE200), with an inner polyvinyl catheter (i.d. 0.5 mm), was inserted into the common carotid artery and advanced into the left ventricle. The catheter position was confirmed by the characteristic pressure pattern. Polyvinyl catheters (i.d. 0.5 mm) were placed in superficial peripheral arteries of both maternal hindlimbs and advanced for 20 cm into the femoral artery.

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The maternal abdomen was opened in the midline, the uterus palpated, and a fetal hindlimb identified. The fetal hindlimb artery and vein were catheterized as has been previously described (10). The uterus and abdomen were closed in layers and the catheters were led via a subcutaneous tunnel to a flank incision where they were maintained in a pouch. The left ventricular catheter was secured under an elastic bandage placed around the neck.

The experiments were performed 2 days after the surgical procedures to allow for recovery from surgical stress. The ewe was placed in a stanchion in the laboratory and permitted to stabilize for 2 hr. The maternal and fetal mean arterial blood pressures were measured with a Statham P23Db transducer placed at the level of the scapulohumeral joint. These parameters were displayed on a Beckman R411 recorder with an EO-18 oscilloscope. Maternal and fetal arterial blood gas analysis was performed prior to and at the conclusion of each experiment.

The EPA_1 was prepared as a 1 mg/ml stock solution in ethanol and stored at 4°. This solution is stable for 6 months at 4° (9). On the days of the experiments dilutions were made from the stock solution and kept on ice until the time of injection.

In five of the experiments, EPA_1 was infused into the mother and the maternal circulatory responses were measured by the radioactive microsphere method. In the other five experiments a bolus injection of the same substance was administered to the fetus and regional blood flows were measured with the same techniques.

In the maternal series, an infusion of 0.5 μg/kg/min was administered via the left ventricular catheter over a 10-min period. Blood flows to individual organs of the mother were measured by injecting radioactive microspheres into the left ventricle while withdrawing an integrated arterial sample from the femoral arterial catheter at a rate of 2.06 ml/min for 1.5 min in a manner previously described (11). A control observation was made of organ blood flows with the initial isotope injection prior to starting the infusion, and a second observation was made just before the conclusion of the 10-min infusion. Microspheres (3M Company and NEN) were 15 μm in diameter and were labeled with one of the following isotopes: ^{46}Sc, ^{57}Co, ^{85}Sr, ^{109}Cd. Each injection contained approximately 1.25 million spheres.

In the fetal series of experiments a bolus of 12.5 μg/kg EPA_1 was injected into the fetal hindlimb vein. Regional blood flows were measured in the control condition (C) and at 5 min after the administration of EPA_1 (T).

At the conclusion of the experiment, the ewe was sacrificed and the kidneys, uterus, and contents were removed. The cotyledons were dissected free from the uterus, and cotyledonary and noncotyledonary tissues were separately assayed. The fetal kidneys were also removed. All tissues were weighed and homogenized with a measured volume of water in a Waring blender. Five aliquots were taken of each homogenate. Each aliquot had a volume of approximately 2 ml and was placed in a preweighed, wide-mouth counting vial which was weighed again. The homogenate reached a height of approximately 1 cm in the vial. Details of these techniques are available in an earlier publication (11). A sample progression was established for each experiment in which standard vials, appropriate to each isotope used in the experiment, were followed by vials containing the blood samples obtained from the integrated arterial sample withdrawal. These were followed by the vials containing the homogenized tissue. All measurements of radioactivity were made with a three-channel, well-type automatic gamma counter (Model 1185 Nuclear Chicago). In experiments in which four isotopes were used, the windows of the gamma counting system were reset and a second pass was made to detect the fourth isotope. The output of the gamma counter was on paper tape which was fed into a Univac 1110 computer via an interactive terminal in our laboratories. Data reduction was performed solving the appropriate algorithms by iterative matrix procedures and programs supplied by our laboratories. The spillover of each isotope in the other channels was determined from the information obtained from

the standard vials, as was the number of counts per minute per microsphere at that time. The data were reduced to the number of spheres per vial of homogenized tissue. The number of microspheres per gram of tissue homogenate was considered to be the average of the number of microspheres per gram observed in the five vials. Blood flow was determined with the equations given by Makowski *et al.* (12). The resistance of the maternal organs was defined as the mean maternal arterial blood pressure divided by the organ blood flow. The resistance of subdiaphragmatic fetal organs was defined as mean fetal arterial pressure minus mean fetal venous pressure, divided by the organ blood flow. Comparisons between the control and test observations were made using a paired *t* test. Results were expressed as mean ± SEM.

Results. Blood gas analysis indicated a mean maternal arterial pH of 7.50 ± 0.12, PCO_2 of 25.8 ± 3 mm Hg and PO_2 of 75.9 ± 8 mm Hg. The blood drawn from the fetal hindlimb artery had a mean pH of 7.43 ± 0.06, PO_2 of 19.8 ± 2 mm Hg, and PCO_2 of 34.6 ± 5 mm Hg.

Maternal series. The dose of EPA_1 chosen, 0.5 μg/kg/min, was similar to the dosage regime used by Wasserman (8) in studying bronchoconstrictor effects in dogs. Observations were obtained in four single and one twin pregnancy. The mean fetal weight was 3.4 ± 0.2 kg. The responses to EPA_1 were as follows. The maternal blood pressure changed from 88 ± 5 to 115 ± 5 mm Hg ($P < 0.002$). This change was apparent within 20–40 sec of commencing the infusion and a hypertensive effect was noted to last for 10–15 min after completion of the infusion. The cotyledonary blood flow was 165 ± 34 (ml/min)/kg fetus before the infusion and 168 ± 24 (ml/min)/kg fetus during the infusion (NS). The renal blood flow was 688 ± 24 ml/min before the infusion and 621 ± 62 ml/min during the infusion (NS). During the infusion of EPA_1 the noncotyledonary blood flow changed from 212 ± 42 to 120 ± 30 ml/min ($P < 0.05$). When resistance was calculated it was found that the cotyledonary resistance did not change during the infusion of EPA_1. The noncotyledonary uterine resistance changed from 0.49 ± 0.08 to 1.19 ± 0.22 PRU ($P < 0.01$), and renal resistance changed from 0.13 ± 0.01 to 0.20 ± 0.03 PRU ($P < 0.025$). These data indicate that EPA_1 causes maternal hypertension and vasoconstriction in the renal and noncotyledonary circulations.

Fetal series. The fetal dose of EPA_1 was chosen empirically to produce a consistent and obvious effect on the fetal blood pressure. Observations were obtained on five fetuses in five sheep. The mean fetal weight was 3.9 ± 0.3 kg. The injection of EPA_1 changed the mean arterial blood pressure from 43 ± 1 to 51 ± 1 mm Hg ($P < 0.01$). The cotyledonary blood flow was 180 ± 22 (ml/min)/kg in the control condition and 143 ± 14 (ml/min)/kg after EPA_1 (NS). EPA_1 caused the membrane blood flow to change from 14 ± 1 to 5 ± 1 (ml/min)/kg ($P < 0.01$) and the renal blood flow to change from 10 ± 0.6 to 7 ± 0.8 (ml/min)/kg ($P < 0.02$). From these data the following resistances were calculated. The cotyledonary resistance changed from 0.06 ± 0.01 to 0.09 ± 0.01 PRU/kg fetus ($P < 0.01$), the membrane resistance changed from 0.84 ± 0.14 to 3.15 ± 0.70 PRU/kg fetus ($P < 0.01$), and the renal resistance changed from 1.18 ± 0.10 to 1.94 ± 0.32 PRU/kg fetus ($P < 0.02$). EPA_1 therefore resulted in fetal hypertension and vasoconstriction in the cotyledonary, membranous, and renal vascular beds.

Discussion. The radioactive microsphere method for measuring organ blood flow is a technique which has been well established (12–14). Sources of error are described by Buckberg *et al.* (13). The primary error is that of having insufficient microspheres in the assay sample. This problem was avoided by counting several samples of the individual tissues, and all the arterial blood samples and tissue samples contained more than 400 microspheres.

The cyclic endoperoxides PGG_2 and PGH_2 are the initial intermediary metabolites produced by the cyclooxygenase catabolism of arachidonic acid. These are the precursor substrates for the production of PGI_2, PGE_2, $PGF_{2\alpha}$, PGD_2, and the thromboxanes. The biological half-life of the endoperoxides is 4–5 min and they have been demonstrated to have powerful

constrictor effects on the vascular and airway smooth muscle which is independent of the stable prostaglandins (8, 15, 16). The methylene derivatives of PGH_2 (cyclic ethers) are called cyclic endoperoxide analogs 1 and 2 (8, 17). These substances are stable in aqueous solution and have been shown to be more potent smooth muscle stimulants than the prostaglandins (8).

In both series of experiments the profound vasoconstrictor effects of EPA_1 were clearly evident. These effects were noted to be consistent in all tissues tested except for the maternal cotyledonary circulation, in which the blood flow and resistance were unaltered. The fetal placental circulation was sensitive to the vasoconstrictor effects of EPA_1 and demonstrated a significant increase in vascular resistance. It may be argued that these differing responses of the two placental circulations were a function of the dosage and method of administering EPA_1 in the two sets of experiments. As all the other organs that were studied demonstrated significant vasoconstriction, the failure of the maternal cotyledonary circulation to respond in a similar fashion would indicate an absolute or relative insensitivity of this particular circulatory bed to the effects of EPA_1. The opposing effects on the two placental circulations is not a unique response. It has been observed in similar experiments using prostaglandin I_2 (6). PGI_2 was noted to cause significant changes in maternal cotyledonary blood flow while fetal cotyledonary blood flow was unaltered. As has been suggested for prostaglandin I_2, the anomalous responses of the placental vasculature to EPA_1 may be the result of an interaction with other vasoactive substances such as circulating catecholamines.

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1. Samuelsson B. Prostaglandin endoperoxides and thromboxanes: Role in platelets and in vascular and respiratory smooth muscle. Acta Biol Med Germ 35:1055–1063, 1976.
2. Rankin JHG, Phernetton TM. Effect of prostaglandin E_2 on ovine maternal placental blood flow. Amer J Physiol 231:754–759, 1976.
3. Rankin JHG, Phernetton TM. Circulatory responses of the near-term sheep fetus to prostaglandin E_2. Amer J Physiol 231:760–765, 1976.
4. Rankin JHG. Role of prostaglandins in the maintenance of the placental circulation. In: Coceani F, Olley PM, eds. Advances in Prostaglandin and Thromboxane Research. New York, Raven Press, Vol 4:pp 261–269, 1978.
5. Rankin JHG, McLaughlin MK. The regulation of the placental blood flows. J Dev Physiol 1:3–30, 1979.
6. Rankin JHG, Phernetton TM, Anderson DF, Berssenbrugge AD. Effect of prostaglandin I_2 on ovine placental vasculature. J Dev Physiol 1:151–160, 1979.
7. Hillier K. Prostaglandins and thromboxanes: Pharmacologic and biosynthetic aspects. Semin Perinatol 2:197–210, 1978.
8. Wasserman MA. Bronchopulmonary pharmacology of some prostaglandin endoperoxides and analogs in the dog. Eur J Pharmacol 36:103–114, 1976.
9. Bundy GL. The synthesis of prostaglandin endoperoxide analogs. Tetrahedron Lett 24:1957–1960, 1975.
10. Rankin JHG, Gresham EL, Battaglia FC, Makowski EL, Meschia G. Measurement of fetal renal inulin clearance in a chronic sheep preparation. J Appl Physiol 32:129–133, 1972.
11. Buss DD, Bisgard GE, Rawlings CA, Rankin JHG. Uteroplacental blood flow during alkalosis in the sheep. Amer J Physiol 228:1497–1500, 1975.
12. Makowski EL, Meschia G, Droegemueller W, Battaglia FC. Measurement of umbilical arterial blood flow to the sheep placenta and fetus in utero. Circ Res 23:623–631, 1968.
13. Buckberg GD, Luck JC, Payne DB, Hoffman JIE, Archie JP, Fixler DE. Some sources of error in measuring regional blood flow with radioactive microspheres. J Appl Physiol 31:598–604, 1971.
14. Rudolph AM, Heymann MA. The circulation of the fetus in utero: Methods for studying distribution of blood flow, cardiac output and organ blood flow. Circ Res 21:163–184, 1967.
15. Tuvemo, T. Role of prostaglandins, prostacyclin, and thromboxanes in the control of the umbilical-placental circulation. Semin Perinatol 4:91–95, 1980.
16. Tuvemo T, Strandberg K, Hamberg M. Contractile action of a stable prostaglandin endoperoxide analogue on the human umbilical artery. Acta Physiol Scand 102:495–496, 1978.
17. Weber PC, Larsson C, Anggard E, Hamberg M, Corey EJ, Nicolaou KC, Samuelsson B. Stimulation of renin release from rabbit renal cortex by arachidonic acid and prostaglandin endoperoxides. Circ Res 39:868–874, 1976.

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The Sex-Related Difference in Perfluorooctanoate Excretion in the Rat[1] (41476)

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Abstract The urinary excretion of perfluorooctanoic acid (PFO) by male and female rats was investigated. Female rats excreted 76 ± 2.7 (SEM)% of a 2-mg dose of nonionic fluorine (as PFO) in the urine in 24-hr whereas male rats excreted only 9.2 ± 3.5% of the dose. The PFO clearance, inulin clearance, net excretion rate of PFO, and the glomerular filtration rate of PFO were measured. The effect of probenecid, an inhibitor of the organic acid transport system, on these measurements was also determined. In female rats the PFO clearance was severalfold greater than the inulin clearance and the clearance of PFO was markedly reduced by probenecid. Conversely, in male rats the PFO clearance was only a fraction of the inulin clearance and was virtually unaffected by probenecid. The data indicate that female rats are able to rapidly eliminate PFO in the urine by an active secretory mechanism which is inhibited by probenecid. In male rats this secretory mechanism is either absent or relatively inactive. This difference in PFO excretion by the male and female may explain the sex-related difference in PFO toxicity in which male rats are more susceptible to high doses than females.

Guy *et al.* have reported the results of attempts to isolate and characterize the compound(s) comprising the nonionic fluorine fraction of human serum (1). They indicate that these compounds are mainly perfluoro fatty acid (C_6-C_8) derivatives and that the major fluorocarbon isolated from human serum resembled perfluorooctanoic acid ($C_7F_{15}COOH$). Since derivatives of perfluorooctanoate (PFO) are widely used commercially it is important to study its behavior in living organisms. In 1980 Ophaug and Singer reported that PFO, administered by stomach intubation to female rats, was rapidly excreted into the urine and by 96 hr only traces remained in the blood (2). Other investigators have fed diets containing 0–1000 ppm of ammonium perfluorooctanoate to rats for a period of 90 days (3). Following an overnight fast the serum of male rats contained 21–49 ppm of PFO whereas the serum of similarly treated females contained 0.15–0.65 ppm. These results confirmed the ability of female rats to rapidly dispose of rather large doses of PFO and illustrate a dramatic sex difference in the metabolic handling of this fluorocarbon by rats. Janssen *et al.* have previously shown that there is also a sex-related difference in the elimination rate of 1-aminocyclohexanecarboxylic acid (ACHC) in rats (4). The principal aim of this investigation was to determine whether there is a sex-related difference in PFO excretion by the rat kidney.

Materials and Methods. Holtzman rats fed rat chow (Purina) and tap water *ad libitum* were employed in all experiments.

Four male and six female rats were administered 2 ml of an aqueous solution containing 2 mg of nonionic fluorine as PFO by stomach intubation. Seven female rats were administered 2 ml of distilled water as controls. The animals were then placed in individual metabolic cages and fed rat chow and tap water. A few crystals of thymol were added to the urine containers to inhibit bacterial growth during the collection period. After 24 hr the animals were sacrificed by cardiac puncture. The blood was allowed to clot and the serum was collected after centrifugation. The volume of the urine collections, including the volume of

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water used to rinse the metabolic cages was recorded. The ionic fluoride content of the serum and urine was determined at pH 5.0 with the fluoride ion-specific electrode (5, 6). The total fluorine content of the serum and urine was determined by the oxygen-bomb reverse extraction technique (7). The nonionic fluorine level of the serum and urine was calculated as the difference between the ionic and total fluorine levels.

For clearance studies of PFO and inulin the rats were anesthetized with Inactin (80–90 mg/kg ip), a barbituric acid derivative which gives a 3- to 4-hr anesthesia. The femoral vein was cannulated for continuous infusion of 5% mannitol in isotonic saline and the femoral artery was cannulated for drawing blood samples. In order to obtain serial collections of urine the urinary bladder was also cannulated. Intravenous (iv) priming doses of 5.2–5.6 mg of [1-^{14}C] ammonium perfluorooctanoate (sp act 0.5 μCi/mg) and 8.8 μg of tritiated inulin (*methoxy*-^{3}H, sp act 114 μCi/mg) were given to each animal. The radiolabeled inulin and PFO in 5% mannitol in isotonic saline was then infused at a rate of 0.21 ml/min. An additional 0.42–0.63 mg/hr of ^{14}C-labeled PFO and 9.6 μg/hr of tritiated inulin was infused during the experiments. After a 45-min equilibration period the first plasma sample was collected for clearance calculations. Urine specimens were collected over 10-min intervals and additional arterial blood samples were obtained at the midpoint of each collection period. When the urine and serum collections for the clearance study were complete probenecid was administered (65–68 mg/kg ip) and, after 20–30 min, additional consecutive 10-min clearance tests were performed to test the effects of probenecid on the organic acid transport system.

In the cumulative excretion study the rats were prepared as described for the clearance tests except that arterial cannulation was not needed. The rats were dosed iv with a mixture of radiolabeled PFO (10–20%) and unlabeled PFO (80–90%). Five percent mannitol in isotonic saline was infused at a rate of 0.081 ml/min and urine specimens were collected over 30-min intervals. The effect of probenecid was assessed by administering 65–68 mg/kg ip at least 30 min prior to the administration of PFO.

The blood samples were centrifuged and 20 μl of plasma was removed for radioactivity determinations. The rest of the plasma sample was transferred to Amicon Centriflo 2100 CF 50 ultrafiltration cones and centrifuged. These membranes retain molecules with a molecular weight greater than 50,000 daltons. Fifty microliters of the ultrafiltrate was also prepared for radioactive determination as was 50 μl of each urine sample.

The ^{3}H (inulin) and/or ^{14}C (PFO) activity in each sample was counted in a dual-channel Packard Tri-Carb liquid scintillation spectrometer. A series of quenched standards and the [^{3}H]inulin and [^{14}C]PFO standards were also counted. The count rates obtained for all samples were corrected for background, quenching (by use of the automatic external standard method), ^{3}H spillover into the ^{14}C counting channel, and ^{14}C spillover in the ^{3}H counting channel. All samples were counted until 20,000 counts had been recorded.

The clearances (C_L) were calculated using

$$C_L \text{ (ml/min/100 g)} = \frac{U \times V}{P \times W},$$

where

U = urinary concentration of the compound (μg/ml),
V = urine flow rate (ml/min),
P = plasma concentration of the unbound compound (μg/ml),
W = animal weight (g)/100.

The net excretion rate (NE) was calculated (μg/min/100 g) using

$$NE = \frac{U \times V}{W},$$

[3] [1-^{14}C]Perfluorooctanoic acid (ammonium salt) was supplied by Minnesota Mining and Manufacturing Company, St. Paul, Minn. 55101.

where

U = urinary concentration of the compound (μg/ml),
V = urine flow rate (ml/min),
W = animal weight (g)/100.

The cumulative excretion percentage was calculated as

$$\% = \frac{U \times VF}{D} \times 100,$$

where

U = urinary concentration of the compound (μg/ml),
VF = total urine volume (ml),
D = total dose of the compound (μg).

The glomerular filtration rate (F) of PFO was obtained from

$$F\ (\mu g/min/100\ g) = P \times C_{L\ (inulin)},$$

where

P = plasma concentration of the unbound compound (μg/ml),
C_L = inulin clearance (ml/min/100 g).

All the tests were performed at least twice unless otherwise stated.

Results. The ionic and nonionic fluorine levels of the serum and the percentage of the dose of nonionic fluorine excreted in the urine 24 hr after administration of 2 mg of nonionic fluorine, as PFO, to male and female rats are presented in Table I. Twenty-four hours after administration of the dose, female rats had excreted 76 ± 2.7% of the dose in the urine and had a mean serum nonionic fluorine level of 0.35 ppm. Although this serum nonionic fluorine level is considerably higher than that observed in female rats which did not receive a dose of nonionic fluorine (0.07 ± 0.02 ppm), male rats had serum nonionic fluorine levels which were much higher (44.0 ± 1.7 ppm) and had excreted only 9.2 ± 3.5% of the dose of nonionic fluorine in the urine. The serum ionic fluoride levels of male and female rats given PFO were not different from that of undosed female rats.

PFO was bound to a similar extent in the plasma of male and female rats. A mean of 97.5 ± 0.25% (SEM, N = 16) of the PFO in the plasma was bound. The results of clearance studies on male and female rats are shown in Table II. In repeated tests it became obvious that there was a crucial difference in PFO clearance and the PFO/inulin clearance ratio between sexes. The PFO clearance in female rats was several times greater than the inulin clearance. The administration of probenecid (65–68 mg/kg ip), which strongly inhibits the renal active secretion system for organic acids (9), dramatically reduced the clearance ratio in female rats. The net excretion of PFO was reduced from 4.6 to 0.13 μg/min/100 g following the administration of probenecid. In male rats, however, the PFO/inulin clearance ratio and the net excretion of PFO were virtually unaffected by probenecid.

TABLE I. SERUM IONIC FLUORIDE AND NONIONIC FLUORINE LEVELS AND URINARY EXCRETION OF NONIONIC FLUORINE 24 HR AFTER ADMINISTRATION[a] OF A 2-MG DOSE OF NONIONIC FLUORINE AS PFO TO MALE AND FEMALE RATS

Sex	Treatment	Serum: Ionic fluoride (ppm)	Serum: Nonionic fluorine (ppm)	Percentage of dose excreted in urine
Female	No dose	0.032 ± 0.003[b] (7)	0.07 ± 0.02 (5)	—
Female	Dosed	0.020 ± 0.003 (6)	0.35 ± 0.11 (6)	76 ± 2.7 (4)
Male	Dosed	0.033 ± 0.003 (4)	44.0 ± 1.7 (4)	9.2 ± 3.5 (4)

[a] Gastric intubation
[b] Mean ± SEM (No. of animals).

TABLE II. EFFECT OF PROBENECID (65–68 MG/KG IP) ON THE PFO/INULIN CLEARANCE RATIO, NET PFO EXCRETION, AND FILTERED PFO IN MALE AND FEMALE RATS

	Female		Male	
	No probenecid	After probenecid	No probenecid	After probenecid
PFO clearance (ml/min/100 g)	5.8[a] (5.5[b]–6.0)	0.11 (0.08–0.14)	0.17 (0.16–0.17)	0.10 (0.09–0.11)
PFO clearance inulin clearance	14.5 (8.2–20.7)	0.46 (0.43–0.48)	0.22 (0.17–0.26)	0.12 (0.11–0.13)
Net PFO excretion (μg/min/mg)	4.6 (3.9–5.2)	0.13 (0.09–0.17)	0.17 (0.13–0.21)	0.12 (0.11–0.12)
Filtered fraction (μg/min/100 g)	0.42 (0.20–0.64)	0.29 (0.21–0.37)	0.75 (0.72–0.77)	1.00 (0.91–1.10)

[a] Mean (range of values) for two animals.
[b] Mean values for each animal of two to four determinations.

Table III presents the data obtained in cumulative excretion studies of PFO in the urine of male and female rats over a 7-hr period. Female rats were observed to excrete 76% of the administered PFO (23–25 mg/kg) whereas male rats excreted only 7.8% of the dose. Intraperitoneal injection of probenecid (65–68 mg/kg) given at least 30 min before administration of the PFO modified the cumulative excretion curve for males only slightly. In female rats, however, probenecid markedly reduced PFO elimination to 11.8%.

Discussion. Griffith and Long have clearly shown a sex-related difference in PFO toxicity in rats being fed PFO in their diet (3). The liver appeared to be the target organ in rats, and males were found to be more susceptible to high doses of PFO than females. The PFO concentration in pooled plasma and liver specimens was considerably higher in male rats than in similarly treated females.

Twenty-four hours after receiving a 2-mg dose of nonionic fluorine (as PFO) male rats had serum nonionic fluorine levels that were more than 100-fold higher than that of similarly treated females (Table I). The serum ionic fluoride levels of male and female rats were not significantly increased following administration of the PFO. This provides good evidence for the metabolic stability of PFO in rats. The conclusion that PFO is metabolically stable in rats is supported by the demonstration of quantitative recovery of nonionic fluorine in the urine and feces of female rats given PFO (2). In addition, Hagen *et al.* have recently demonstrated the accumulation of PFO in the serum of male rats given a single oral dose of 1H,1H,2H,2H-perfluorodecanol (8). In contrast to female rats which excreted 76 ±

TABLE III. EFFECT OF PROBENECID (65–68 MG/KG IP) ON THE CUMULATIVE URINARY EXCRETION OF PFO IN MALE AND FEMALE RATS[a]

	Female		Male	
Hours	No probenecid	After probenecid	No probenecid	After probenecid
1	21.6 (20.2–22.9)[a]	1.1 (1.1–1.1)	1.4 (0.9–1.8)	1.0 (0.9–1.1)
2	36.3 (32.7–39.9)	2.5 (2.2–2.7)	2.4 (1.6–3.1)	1.7 (1.5–1.8)
3	46.1 (38.6–53.5)	3.6 (3.1–4.1)	3.4 (2.4–4.3)	2.4 (2.2–2.6)
4	56.5 (49.5–63.4)	5.3 (4.0–6.6)	4.5 (3.5–5.4)	3.1 (2.8–3.3)
5	65.7 (59.3–72.0)	7.2 (4.8–9.6)	5.7 (4.9–6.4)	3.7 (3.3–4.0)
6	71.9 (64.8–78.9)	9.2 (5.8–12.6)	6.8 (6.3–7.3)	4.4 (3.9–4.8)
7	76.2 (68.9–83.5)	11.8 (6.6–17.0)	7.8 (7.3–8.2)	5.0 (4.4–5.5)

[a] Results are percentage of dose excreted.
[b] Mean (range of values) for two animals.

2.7% of the PFO dose in the urine after 24 hr, male rats excreted only 9.2 ± 3.5% of the dose (Table I). These data indicate that the sex-related difference in PFO toxicity in rats (3) is due to the relatively slow urinary excretion of PFO in male rats.

Since inulin is excreted only by glomerular filtration and not actively secreted in renal tubuli, the observation of PFO/inulin clearance ratios that were substantially greater than 1.0 for female rats (Table II) provides evidence that, in the female rat, PFO is excreted in part by an active secretion mechanism. The fact that probenecid rapidly decreases the PFO/inulin clearance ratio from 14.5 to 0.46 strongly supports this conclusion. Since the PFO/inulin clearance ratio for male rats was less than 1.0 and not significantly altered by the administration of probenecid it appears that active tubular secretion of PFO in males either does not occur or occurs at an insignificant rate. Additionally, both the female rat after receiving probenecid and the male rat throughout the clearance studies had lower PFO than inulin clearance. This indicates that there is partial tubular reabsorption of PFO in both sexes. Janssen *et al.* have shown that there is a sex-related difference in the tubular reabsorption of 1-aminocyclohexanecarboxylic acid (ACHC) in the rat kidney (4). ACHC is not bound to plasma proteins whereas in the present study 97.5 ± 0.25% (SEM) of the PFO was bound. Thus the excretion of ACHC by glomerular filtration occurs to a much greater extent than PFO. Our data indicate that there is a sex-related difference in the active secretion of PFO. It is therefore possible that female rats also actively secrete ACHC to a greater extent than male rats.

The cumulative urinary excretion data in Table III illustrates the striking sex-related difference in PFO elimination. At doses of 23–25 mg/kg male rats, because of a limited or completely inactive secretion mechanism, are able to net-excrete in 7 hr only about 10% of the amount of PFO excreted by females. Although probenecid had little effect on the cumulation excretion curve for males a low level of active tubular secretion of PFO cannot be ruled out. It is possible that probenecid, as an acid, slightly increases the tubular reabsorption of PFO and partially masks the active secretion.

Ophaug and Singer found that female rats excreted 61% of the administered dose of nonionic fluorine (PFO) in 8 hr (2). The more rapid excretion (76% in 7 hr) observed for female rats in the cumulative excretion studies (Table III) is probably due to the fact that the animals in the present study were infused with 5% mannitol in isotonic saline during the excretion period and that the PFO was administered iv rather than by gastric intubation. After the administration of probenecid to female rats the rate of excretion of PFO decreased dramatically and after 1 hr there was no difference in PFO elimination between sexes. Griffith and Long observed that rhesus monkeys do not exhibit a sex-related difference in the elimination of PFO (3). Serum PFO levels (following an overnight fast) ranged from 45 to 71 ppm for males receiving 3 or 10 mg PFO/kg/day as compared to levels of 50 to 79 ppm for corresponding females. The high serum levels of PFO found in both sexes of rhesus monkey closely reflect the situation found in male rats in that they appear to eliminate a dose of PFO rather slowly. One might speculate, therefore that the probenecid-sensitive active secretory system we have observed in female rats is absent or inactive in both sexes of the rhesus monkey.

Based upon these data it is concluded that the female rat possesses an active secretory mechanism which rapidly eliminated PFO from the body. This secretory mechanism is lacking or relatively inactive in male rats and accounts for the greater toxicity of PFO in male rats.

1. Guy WS, Taves DR, Brey WS. Organic fluorocompounds in human plasma: Prevalence and characterization. In: Filler R, ed. Biochemistry Involving Carbon-Fluorine Bonds. ACS Symposium Series No. 28. Washington, DC, Amer Chem Soc, Vol 7:p117, 1976.
2. Ophaug RH, Singer L. Metabolic handling of perfluorooctanoic acid in rats. Proc Soc Exp Biol Med 163:19–23, 1980.

3. Griffith FD, Long JE. Animal toxicity studies with ammonium perfluorooctanoate. Amer Ind Hyg Assoc J 41:576–583, 1980.
4. Janssen FW, Young EM, Ruelius HW. Effect of sex hormones on the disposition in rats of 1-aminocyclohexane carboxylic acid, a metabolite of a semisynthetic penicillin. Drug Metab Dispos 4:540–546, 1976.
5. Singer L, Armstrong WD. Determination of fluoride in ultrafiltrates of sera. Biochem Med 8:415–422, 1973.
6. Singer L, Armstrong WD, Vogel JJ. Determination of fluoride content of urine by electrode potential measurements. J Lab Clin Med 74:354–458, 1969.
7. Venkateswarlu P. Determination of total fluorine in serum and other biological materials by oxygen bomb and reverse extraction techniques. Anal Biochem 68:512–521, 1975.
8. Hagen DF, Belisle J, Johnson JD, Venkateswarlu P. Characterization of fluoridated metabolites by a gas chromatographic-helium microwave plasma detector—The biotransformation of 1H,1H,2H,2H-perfluorodecanol to perfluorooctonate. Anal Biochem 118:336–343, 1981.
9. Weiner IM, Mudge GH. Renal tubular mechanisms for excretion of organic acids and bases. Amer J Med 36:743–762, 1964.

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Localization of Radiolabeled Antibody in SVT2 Tumor Increases with Immunosuppression of the Host (41477)

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Abstract. The localization of radiolabeled tumor-specific antibodies to an SV40-transformed mouse tumor was analyzed in immunosuppressed (X-ray and cortisone) and nonimmunosuppressed mice. (C57Bl/6 × Balb/c)F_1 mice were immunized with the SVT2 tumor of Balb/c origin. Radiolabeled antibody was isolated from ^{125}I-labeled immune gamma globulin by adsorption onto SVT2 cells, and elution from these cells using citrate buffer. The radiolabeled antibodies were injected into normal (C57Bl/6 × Balb/c)F_1 mice. The purified antibodies present in this serum bound specifically *in vitro* to SV40-transformed cell lines. *In vivo*, the ^{125}I-labeled antibodies localized preferentially in the SVT2 tumor in immunosuppressed mice. Significantly less ^{125}I-labeled antibody localized in the SVT2 tumor in nonimmunosuppressed mice. The localization of ^{125}I-labeled antibody in SVT2 tumor in immunosuppressed mice was reduced significantly by passive administration of anti-SVT2 serum.

Cancer diagnosis or therapy using a tumor localizing radiolabeled antibody has been the goal of several groups of investigators (1–7). Most of the work has been done in xenogeneic systems, which require exhaustive absorptions of immune serum with normal cells to obtain a more tumor-specific serum. It would be desirable to work in a syngeneic system in which the only antibody response elicited is that against unique antigens on the tumor cell surface. Simian virus 40 (SV40)-transformed mouse cells possess an antigen specific for SV40-induced tumors that is absent in non-SV40-induced tumors (8–11). It has been shown that immunization of (C57Bl/6 × Balb/c)F_1 mice with syngeneic SV40 tumor (SVT2) elicits antibodies reacting against the SV40 tumor-associated cell surface antigen (10, 11). We chose this syngeneic system to avoid the cross-reactivity problems often associated with the use of tumor localizing antibodies prepared from xenogeneic serum (2).

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Two possible limitations in the use of radiolabeled antibodies are the presence of circulating tumor antigens and host antibody. Tumor antigens which are shed from the solid tumor may combine with injected radiolabeled antibody, and the complexes cleared. However, tumor localization by radiolabeled antibodies has occurred even in the presence of high levels of circulating CEA (4, 5, 12). Antibodies produced by the host to its growing tumor may bind to the tumor, masking the binding sites from the labeled antibody (13, 14). Here we report that immunosuppression of the host prior to transplantation of an SV40-transformed mouse tumor led to significantly increased tumor localization of radiolabeled tumor-specific antibodies. This tumor localization was blocked by passive administration of immune serum.

Materials and Methods. *Mice and immunization.* Eight-week-old male (C57Bl/6 × Balb/c)F_1 mice (Animal Genetics and Production Branch, National Cancer Institute, Frederick, Md.) genetically identical to those used by Ting (10, 11) were immunized with the SVT2 tumor. The SVT2 and SVA31 C14 lines of Balb/c embryo cells

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transformed by SV40 were obtained frozen from a tumor bank (Biotech Research Laboratories, Inc., Rockville, Md.) recommended by Dr. C. C. Ting at the NCI. They were grown as monolayers in 150-cm^2 tissue culture flasks (Corning Glass Works, Corning, N.Y.) in RPMI 1640 medium containing 5% heat-inactivated calf serum (Grand Island Biological Co., Grand Island, N.Y.), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 m*M* L-glutamine at 37° in a humidified atmosphere with 5% CO_2–95% air. The cells were harvested by exposure to 0.1% trypsin–10 m*M* EDTA in PBS, pH 7.2. The mice were immunized with the SVT2 tumor by inoculation of 1×10^7 cells into the footpad. Antisera were collected by cardiac puncture from mice with progressively growing tumors 4 to 6 weeks after tumor inoculation.

Isotopic antiglobulin technique. Sera were assayed for antibody activity by an isotopic antiglobulin technique (15). The antiglobulin used in this procedure was the IgG fraction of sheep anti-mouse IgG (Cappel Laboratories, Cochranville, Pa.) radiolabeled with ^{125}I (Amersham Corp., Chicago, Ill.) at a specific activity of 0.25 mCi/mg by the iodine monochloride method (16). Briefly, 5×10^5 target cells in 0.1 ml HBSS were incubated first with 0.1 ml of a 1:50 dilution of antiserum or normal serum for 30 min at room temperature. The cells were washed four times with 1 ml HBSS containing 10% γ-globulin-free newborn calf serum (Grand Island Biological Co., Grand Island, N.Y.). A second incubation with 10 μl ^{125}I-labeled sheep anti-mouse Ig containing approximately 3×10^6 cpm was done at 0° for 15 min, and the cells were washed seven times. All tests were done in duplicate. The variation among duplicates did not exceed 10%. Activities of the antisera are expressed as an absorption ratio (AR):

$$AR = \frac{\text{^{125}I cpm on cells incubated with mouse antiserum + ^{125}I-antiglobulin}}{\text{^{125}I cpm on cells incubated with normal mouse serum + ^{125}I-antiglobulin}}.$$

A reaction was considered positive when the AR was ≥ 3.

Gamma globulin preparation. Gamma globulin was prepared from pooled anti-SVT2 antiserum by three precipitations with neutral 50% saturated ammonium sulfate. The final precipitate was dissolved in a volume of PBS equal to 40% of the original serum volume. Normal gamma globulin was prepared by ammonium sulfate precipitation of (C57Bl/6 × Balb/c)F_1 serum; the final precipitate was dissolved in a volume of PBS equal to 27% of original serum volume.

Antibody purification and radiolabeling. The immune gamma globulin was enriched before labeling by affinity purification using a modified version of the procedure of Ehrlich and Witz (17). SVT2 cells were washed twice with PBS, and resuspended in serum-free RPMI 1640 medium at a concentration of 2.2×10^7 cells/ml. Aliquots containing 1.35 ml of the SVT2 cell suspension and 0.15 ml anti-SVT2 serum were combined in 15-ml conical test tubes (Falcon Plastics, Los Angeles, Calif.). These were incubated in an ice bath for 1 hr with occasional shaking. After incubation, the cells were pelleted and washed three times with PBS. Antibody remaining adsorbed to the packed cells was eluted by resuspending each pellet in 0.1 ml of 0.1 *M* citrate buffer, pH 3.5, for 15 min. The supernatants were pooled, neutralized with 1 *N* NaOH, and dialyzed against one liter of PBS overnight. The entire procedure—absorption, washes, elution, and dialysis—was carried out at 4°. The dialyzed eluate was added to 1.5 mg of anti-SVT2 Ig to produce "enriched" immune Ig.

Two preparations of enriched immune Ig were radiolabeled by the iodine monochloride method (16). Gamma globulin, 1.5 mg, was dialyzed against one liter of borate buffer (0.20 *M* boric acid, 0.16 *M* NaCl, 0.04 *M* NaOH, pH 8.0) overnight before iodination. ^{125}I (15 mCi) and 4 eq of ICl were used to label the immune gamma globulins to a specific activity of 4.5 and 5.3 mCi/mg. Normal (C57Bl/6 × Balb/c)F_1 serum was used as a protective protein for the labeled preparations at 20% of the final

volume. One milligram of normal Ig was labeled in a similar fashion with 1 mCi of ^{131}I and 4 eq of ICl.

The radiolabeled antibody preparations were once affinity-purified by the procedure described above. Aliquots containing 2.25 ml of the SVT2 cell suspension and 0.25 ml ^{125}I-labeled enriched anti-SVT2 Ig were combined and incubated in an ice bath 1 hr. Washes, elution, and dialysis have been detailed above. The ^{125}I-labeled once affinity-purified antibodies were further purified by *in vivo* screening (18). Approximately 400,000 and 700,000 cpm of Preparations I and II of once-absorbed and eluted antibody in 0.5 ml PBS were each injected ip into four normal mice. The mice were bled 24 hr later by cardiac puncture. Approximately 15% of the injected counts were recovered in the serum. ^{131}I-labeled normal Ig was also *in vivo* screened. Approximately 1.5×10^6 cpm of ^{131}I-labeled normal Ig was injected ip into five normal (C57Bl/6 × Balb/c)F_1 mice. Approximately 3% of the counts injected were recovered in the serum 24 hr later.

A portion of the enriched immune gamma globulin was passed over a protein A–sepharose column (19), and the individual IgG fractions were radiolabeled with ^{125}I. Each fraction was affinity-purified with SVT2 cells and *in vivo* screened in normal (C57Bl/6 × Balb/c)F_1 mice, and tested for binding to finely minced tumor pieces taken from immunosuppressed and nonimmunosuppresed mice.

In vivo distribution studies. The *in vivo* distribution of the radiolabeled purified anti-SVT2 antibodies was studied in nonimmunosuppressed and immunosuppressed mice. The immunosuppressed mice consisted of a group of 11 (C57Bl/6 × Balb/c)F_1 mice treated with 400 R of whole-body radiation from a General Electric 220-keV Maximar X-ray machine the day before tumor transplantation, and given a 0.25 mg sc dose of cortisone acetate on alternate days beginning with the day of tumor transplant. SVT2 tumors were started in the right flank in these mice with trocar transplants of SVT2 tumor maintained in irradiated (C57Bl/6 × Balb/c)F_1 mice. MOPC-315 tumors were started in the left flank in these mice with trocar transplants of MOPC-315 tumor maintained in Balb/c AnN (The Charles River Breeding Laboratories, Inc., Wilmington, Mass.) mice. The five nonimmunosuppressed mice received no cortisone or X-ray. The tumors were started in these mice at the same time they were transplanted into the immunosuppressed mice. Nineteen days after transplant, four tumor-bearing immunosuppressed mice received 0.3 ml of unlabeled (C57Bl/6 × Balb/c)F_1 anti-SVT2 serum ip. The next day, these four mice (Group III) and seven other immunosuppressed mice that were not given unlabeled antiserum (Group II) received ip a mixture of 0.01 μCi of Preparation I of ^{125}I-labeled enriched once affinity-purified/*in vivo*-screened antibody and 0.02 μCi ^{131}I-labeled, *in vivo*-screened normal (C57Bl/6 × Balb/c)F_1 gamma globulin. Group I containing five nonimmunosuppressed mice received 0.01 μCi of Preparation II of ^{125}I-labeled enriched once affinity-purified/*in vivo* screened antibody and 0.02 μCi ^{131}I-labeled, *in vivo* screened normal gamma globulin. Forty-eight hours after injection, mice were bled by cardiac puncture, dissected, and the tissues counted. For a week prior to injection of radiolabeled antibody and until dissection, the mice were given water containing 6×10^{-4} *M* KI to block thyroid uptake of iodine that could be freed by the catabolism of the labeled gamma globulins.

Results. High absorption ratios against SVT2 cells were obtained when testing serum from all groups of immunized mice. The sera were pooled, and the effect of concentration of antiserum on the absorption ratio obtained with SVT2 tumor cells indicated a titer of 1:8192. Table I demonstrates that the pooled anti-SVT antiserum reacted with SVT2 and SVA31 C14 cells, but did not bind to MOPC-315 myeloma cells of Balb c origin, P-815-X2 mastocytoma cells of DBA 2 origin, RIF-1 radiation-induced fibrosarcoma cells of C3H HeJ origin, B16-F1 melanoma cells of C57Bl 6 origin, or SCK spontaneous tumor cells of A J origin.

The specificity of the ^{125}I-labeled anti-

TABLE I. ABSORPTION RATIOS OF (C57BL/6 × BALB/C)F_1 ANTI-SVT2 ANTISERUM WITH VARIOUS TUMOR CELLS

Serum[a]	Target cells	cpm of antiserum	cpm of normal serum[b]	AR[c]
Pooled progressor	SVT2	27,961	1157	24.2
	SVA31 C14	18,327	1230	14.9
	MOPC-315	5,113	4096	1.3
	P-815-X2	1,192	1268	0.9
	RIF-1	1,141	1018	1.1
	B16-F1	1,281	984	1.3
	SCK	1,680	1131	1.5

[a] A 1:50 dilution of mouse serum was incubated with 5×10^5 target cells for 30 min at room temperature. The cells were then washed four times, incubated with 10 μl of the ^{125}I-antiglobulin for 15 min at 0°, and then washed seven times. Each determination was performed in duplicate.

[b] Control serum was normal (C57Bl/6 × Balb/c)F_1 at a 1:50 dilution.

[c] Absorption ratio = ^{125}I cpm antiserum/^{125}I cpm normal serum (average of duplicate samples).

TABLE II. *IN VITRO* BINDING OF ^{125}I-LABELED (C57BL/6 × BALB/C)F_1 ANTI-SVT2 ANTIBODY AT VARIOUS STAGES OF PURIFICATION

Purification stage	Binding (%) to tumor cells[a] SVT2	P-815-X2
1. Enriched ^{125}I-gamma globulin		
Preparation I	1.2	—
Preparation II	0.8	0.8
2. Enriched, once absorbed and eluted		
Preparation I	42.0	6.7
Preparation II	43.7	3.5
3. Enriched, once absorbed and eluted/*in vivo* screened		
Preparation I	37.2	0.2
Preparation II	46.2	0.3
4. Enriched, twice absorbed and eluted		
Preparation I	54.3	3.3

[a] SVT2 tumor cells or P-815-X2 control cells were suspended in RPMI 1640 medium containing 5% calf serum at a concentration of 1.7×10^7 cells/ml. Six-tenths ml of the cell suspension (10^7 cells) was placed into 12 × 75-mm glass tubes, and a volume of antibody containing a minimum of 3000 cpm was added to duplicate tubes. The tubes were incubated at 37° for 1 hr with occasional shaking, and then counted in a Beckman Gamma 7000 counting system to determine the total cpm added. After three washes with 1 ml of RPMI 1640 medium containing 10% calf serum, the tubes were counted again. Mean values are shown. The variation between duplicate tubes did not exceed 10%.

SVT2 antibody was tested at various stages of purification by an *in vitro* binding assay, as detailed in Table II legend. Table II shows the specific binding of enriched, affinity-purified, and affinity-purified/*in vivo*-screened antibody preparations. The affinity purification brought the specific binding to SVT2 cells from 1.2 to 42.0–43.7%. *In vivo* screening of affinity-purified antibodies did not increase the binding to SVT2 cells, but did reduce the nonspecific binding to P-815-X2 mastocytoma cells essentially to zero.

The dose–response effect of immunosuppression and change in titer of circulating host antibody to SVT2 is shown in Table III. We used techniques of analysis of variance and regression to determine the affect of radiation and cortisone on the AR. There is a significant slope (linear effect) of radiation on the absorption ratio for both the with- and without-cortisone groups of mice. However, the two slopes are significantly different with the steeper slope associated with the cortisone group.

The tissue to blood ratios of ^{125}I-labeled anti-SVT2 antibody and ^{131}I-labeled normal gamma globulin in the three groups of tumor-bearing mice are shown in Table IV. The SVT2 tumor load (mean ± SE) in

TABLE III. EFFECT OF IMMUNOSUPPRESSION ON CHANGE IN TITER OF CIRCULATING HOST ANTIBODY IN MICE BEARING SVT2 TUMORS

Treatment	No. of mice in group	AR[a]
0 R	3	5.1 ± 0.4
0 R and cortisone	4	5.2 ± 0.4
200 R	4	4.0 ± 0.8
200 R and cortisone	3	3.6 ± 0.3
400 R	4	3.7 ± 0.5
400 R and cortisone	6	1.6 ± 0.4

[a] Mean ± SE obtained by dividing counts of ^{125}I-labeled sheep anti-mouse Ig bound to SVT2 cells incubated with serum obtained from individual mice in the different treatment groups, 3 weeks after sc transplantation of the SVT2 tumor, by the counts bound to SVT2 cells incubated with normal serum.

TABLE IV. TISSUE TO BLOOD RATIOS OF ^{125}I-LABELED ANTI-SVT2 ANTIBODY AND ^{131}I-LABELED NORMAL GAMMA GLOBULIN IN TUMOR-BEARING MICE INJECTED WITH ANTI-SVT2 SERUM[a]

	Group I (Nonimmunosuppressed)		Group II (Immunosuppressed)		Group III (Immunosuppressed + antiserum)	
Tissue	^{125}I-Antibody	^{131}I-Normal gamma globulin	^{125}I-Antibody	^{131}I-Normal gamma globulin	^{125}I-Antibody	^{131}I-Normal gamma globulin
SVT2 tumor	0.53 ± 0.12	0.30 ± 0.04	2.17 ± 0.23	0.55 ± 0.06	0.88 ± 0.11	0.55 ± 0.08
MOPC-315 tumor	0.43 ± 0.11	0.33 ± 0.07	0.66 ± 0.07	0.52 ± 0.03	0.32 ± 0.02	0.34 ± 0.01
Spleen	0.18 ± 0.02	0.17 ± 0.01	0.11 ± 0.04	0.19 ± 0.04	0.15 ± 0.03	0.18 ± 0.03
Liver	0.23 ± 0.01	0.19 ± 0.01	0.24 ± 0.01	0.21 ± 0.01	0.20 ± 0.02	0.16 ± 0.01
Heart	0.27 ± 0.02	0.26 ± 0.02	0.34 ± 0.07	0.23 ± 0.03	0.18 ± 0.04	0.25 ± 0.01
Lung	0.39 ± 0.02	0.40 ± 0.02	0.43 ± 0.10	0.45 ± 0.04	0.30 ± 0.03	0.42 ± 0.08
Kidney	0.28 ± 0.02	0.26 ± 0.01	0.31 ± 0.07	0.27 ± 0.02	0.24 ± 0.04	0.25 ± 0.03
Muscle	0.12 ± 0.02	0.12 ± 0.01	0.45 ± 0.13	0.52 ± 0.12	0.50 ± 0.15	0.42 ± 0.13
Skin	0.51 ± 0.09	0.41 ± 0.08	1.30 ± 0.24	1.17 ± 0.20	0.95 ± 0.12	0.87 ± 0.17
Small intestine	0.15 ± 0.04	0.14 ± 0.02	0.08 ± 0.02	0.11 ± 0.01	0.08 ± 0.03	0.08 ± 0.02

[a] Groups I–III consist of five, seven, and four mice, respectively. Groups II and III were immunosuppressed with radiation and cortisone and were inoculated with Preparation I of ^{125}I-labeled anti-SVT2 antibody and ^{131}I-labeled normal gamma globulin 20 days after tumor transplant. Group I was nonimmunosuppressed and received Preparation II of ^{125}I-labeled anti-SVT2 antibody and ^{131}I-labeled normal gamma globulin 25 days after tumor transplant. Mice were killed 48 hr after injection. Results are expressed as mean tissue to blood ratio (calculated on an individual animal basis and averaged for each group) ± SE.

Groups I–III was 0.31 ± 0.12, 0.44 ± 0.20, and 0.36 ± 0.11 g, respectively. The difference in SVT2 tumor load between any of the groups was statistically insignificant. There was significantly less ^{125}I-labeled antibody localized in the SVT2 tumor in Group I of nonimmunosuppressed mice compared with Group II of immunosuppressed mice, determined by two sample *t* test ($P < 0.01$). The localization of ^{131}I-labeled normal gamma globulin was lower in SVT2 tumor and skin in the nonimmunosuppressed mice (Group I, $P < 0.01$). The localization of ^{125}I-labeled antibody in SVT2 tumor in immunosuppressed mice (Group II) was reduced by passive administration of 0.3 ml anti-SVT2 serum (Group III, $P < 0.01$). The localization of ^{125}I-labeled antibody and ^{131}I-labeled normal gamma globulin was lower in the MOPC-315 tumor in mice injected with anti-SVT2 serum (Group III) compared with immunosuppressed mice (Group II, $P < 0.01$), but was similar to that seen in nonimmunosuppressed mice (Group I). The sera from the individual animals in the three groups were tested by the isotopic antiglobulin technique. The mean values for Groups I–III were 6.6, 1.8, and 5.6, respectively, indicating the presence of host-anti-SVT2 antibody in the nonimmunosuppressed mice. Table V shows the *in vitro* binding of the radiolabeled IgG antibody fractions to crude cellular preparations made by finely mincing SVT2 tumors taken from immunosuppressed and nonimmunosuppressed mice. There was lower binding to tumors taken from nonimmunosuppressed mice. Exposure of the cellular preparation made from tumor grown in nonimmunosuppressed mice to citrate buffer, pH 3.5, did not result in an eluate that showed antibody activity by the isotopic antiglobulin technique. Incubation of the cellular preparation with ^{125}I-labeled sheep anti-mouse IgG did not result in greater binding of the antiglobulin than that seen with a cellular preparation made from tumors grown in immunosuppressed mice.

Discussion. The possibility of using radiolabeled antibodies in syngeneic tumor models has been investigated in several other laboratories. Witz *et al.* (20) immunized syngeneic mice with Moloney virus-induced lymphomas. When labeled affinity-purified antibodies were injected into animals bearing the tumor, essentially the only organ which showed preferential uptake of the labeled antibody was the spleen. There was no such spleen fixation in normal mice, suggesting that the spleens from tumor-bearing mice contained tumor cells, antigen, or virus. De Vaux Saint Cyr (21) found no tumor localization when studying a radiolabeled syngeneic antibody against a hamster SV40 tumor. This lack of localization may have been the result of a disappearance of the virus-induced antigens

TABLE V. *In Vitro* Binding of Anti-SVT2 Immunoglobulin Fractions to SVT2 Cellular Preparations

	Binding (%)[a]		
	SVT2 cells in culture	SVT2 tumor pieces from immunosuppressed mice	SVT2 tumor pieces from nonimmunosuppressed mice
^{125}I-IgG$_1$	29.5	26.9	11.7
^{131}I-Normal γ-globulin	—	3.2	1.9
^{125}I-IgG$_{2a}$	25.8	21.8	11.3
^{131}I-Normal γ-globulin	—	2.9	3.3
^{125}I-IgG$_{2b}$	15.8	22.9	5.5
^{131}I-Normal IgG$_{2b}$	—	4.8	3.8

[a] 1×10^7 SVT2 cells maintained in tissue culture or 0.1 g of a crude cellular preparation made by finely mincing tumors taken from immunosuppressed (400 R X-ray and cortisone) or nonimmunosuppressed mice, were incubated with a minimum of 2000 cpm of radiolabeled antibody or normal γ-globulin, and the cpm bound/cpm added initially (average of duplicate samples) determined.

from SV40 tumor cells *in vivo*. These antigens reappeared after the cells were cultured *in vitro*. In contrast, our results show that ^{125}I-labeled tumor-specific antibodies can be prepared from the sera of mice with progressively growing SVT2 tumors, and that when passively transferred these antibodies localize specifically in SVT2 tumors growing in immunosuppressed (X-ray and cortisone) mice. This localization was blocked by passive administration of anti-SVT2 serum or by nonimmunosuppression of the host. We used the F_1 hosts and SVT2 tumor to exactly duplicate the model and results reported by Ting (10, 11). The specificity of the antisera obtained from F_1 hosts was tested by absorption experiments. Their reactivity could only be abrogated by absorption with various SV40-transformed cells (10, 11). To assure that we were dealing with a tumor-specific antigen and not a histocompatibility antigen, we obtained the SVT2 tumor frozen from a tumor bank. In addition, the F_1 mice were from the same source at the NCI, where a new colony is started each year to prevent genetic drift. Finally, the antibody did not show any cross-reactivity with other tumors of C57Bl/6 or Balb/c background (Table I).

The results of the present study suggest that the presence of antibody, produced for example by an intact immune system, can block the localization of radiolabeled syngeneic tumor-specific antibodies to the SV40 tumor-specific cell surface antigen *in vivo*. The findings that immunosuppressed mice given anti-SVT2 serum had significant free antibody in the circulation and that the radiolabeled antibody fractions showed lower binding to tumors taken from nonimmunosuppressed mice, suggest that deposition of unlabeled antibody in tumors masks or modulates (21) antigenic sites on the SV40 tumor cell surface and prevents their recognition by radiolabeled tumor localizing antibodies. Witz *et al.* (22) reported that irradiation (400 rad) of the host prior to MDAY methylcholanthrene-induced sarcoma cell inoculation caused a decreased coating of tumor cells with immunoglobulin. Ran *et al.* (23) eluted cytotoxic antitumor antibodies from SEYF-a tumor cells using low pH buffers. However, acid elution gave no direct evidence of the presence of antibody on the SVT2 tumor *in situ*. Izzo *et al.* (24) reported that horse anti-rat lymphocyte serum blocked the localization in allogeneic skin grafts of radiolabeled histocompatibility antibody.

In Table IV, there was an increase of ^{131}I-labeled normal gamma globulin localization in SVT2 tumor in the immunosuppressed and immunosuppressed + antiserum groups relative to the nonimmunosuppressed group. This can probably be explained by the well-known increase in skin vascular permeability following X-ray (25). The localization of normal gamma globulin in the SVT2 tumor was not reduced in the immunosuppressed mice given antiserum (Group III) compared with immunosuppressed mice (Group II), but was reduced somewhat in muscle and skin. The reason for this reduction is not clear. However, it should be kept in mind that these differences involve tissue to blood ratios that are quite uniform and in most instances considerably less than 1, whereas the SVT2 tumor to blood ratio is greater than 2 at 48 hr in Group II. Ballou *et al.* (1, 26) reported that radiolabeled monoclonal antibody localized preferentially in a mouse teratinocarcinoma in syngeneic mice, with a tumor to blood ratio of 1.3 at 48 hr after injection. This ratio increased to 15 at 5 days after injection due to the rapid elimination of the IgM antibody from the blood.

Present studies using the SVT2 model have limitations in replicating what is presently being attempted clinically. First, to date, most radiolabeled antisera have been of heterologous derivation, goat and rabbit into man rather than comparable to the experimental situation of murine antibody into a murine host. In addition, most of the antigens studied in man have been tumor associated, i.e., in high concentration but not unique, nor immunogenic. HCG,[2] CEA, ferritin and AFP are generally not consid-

[2] Abbreviations used: HCG, human chorionic gonadotropin; CEA, carcinoembryonic antigen; AFP, α-fetoprotein.

ered immunogenic to the host nor have they been clearly demonstrated to produce host autoantibody.

The results suggest that a possible limitation in the use of radiolabeled antibodies for cancer diagnosis and therapy might be the production by the host of antibodies that bind to antigen sites on the tumor cell surface and block binding of labeled antibody. This would only be relevant to those antigens which induce autoantibody and only then if the heterologous-derived antibody would be similarly blocked. However, there is a high probability that monoclonal antibodies produced by hybridomas of human origin will eventually be used in cancer diagnosis and therapy, so that the results in mouse systems will be of importance in indicating the directions human studies should take.

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1. Ballou B, Levine G, Hakala TR, Solter D. Tumor location detected with radioactively labeled monoclonal antibody and external scintigraphy. Science 206:844–847, 1979.
2. Buchsbaum DJ, Walker PC, Johnson EA. Distribution of radiolabeled alloantibodies in mice bearing 3-methylcholanthrene-induced sarcomas. Cancer Res 39:3363–3368, 1979.
3. Ghose T, Norvell ST, Aquino J, Belitsky P, Tai J, Guclu A, Blair AH. Localization of ^{131}I-labeled antibodies in human renal cell carcinomas and in a mouse hepatoma and correlation with tumor detection by photoscanning. Cancer Res 40:3018–3031, 1980.
4. Goldenberg DM, Kim EE, DeLand FH, Bennett S, Primus FJ. Radioimmunodetection of cancer with radioactive antibodies to carcinoembryonic antigen. Cancer Res 40:2984–2992, 1980.
5. Mach J-P, Buchegger F, Forni M, Ritschard J, Berche C, Lumbroso J-D, Schreyer M, Girardet C, Accolla RS, Carrel S. Use of radiolabelled monoclonal anti-CEA antibodies for the detection of human carcinomas by external photoscanning and tomoscintigraphy. Immunol Today 2:239–249, 1981.
6. Order SE, Klein JL, Ettinger D, Alderson P, Siegelman S, Leichner P. Use of isotopic immunoglobulin in therapy. Cancer Res 40:3001–3007, 1980.
7. Pressman D. The development and use of radiolabeled antitumor antibodies. Cancer Res 40:2960–2964, 1980.
8. Ting C-C, Herberman RB. Detection of tumor-specific cell surface antigen of Simian virus 40-induced tumors by the isotopic antiglobulin technique. Int J Cancer 7:499–506, 1971.
9. Ting C-C, Rodrigues D, Herberman RB. Expression of fetal antigens and tumor-specific antigens in SV40-transformed cells. II. Tumor transplantation studies. Int J Cancer 12:519–523, 1973.
10. Ting C-C, Ortaldo JR, Herberman RB. Serologic analysis of the antigenic specificities of Simian virus 40-transformed cells and their relationship to tumor-associated transplantation antigen. J Nat Cancer Inst 52:815–821, 1974.
11. Ting C-C. Detection of anti-tumor antibody in virally induced tumors and its relationship to tumor growth. Int J Cancer 18:205–215, 1976.
12. Primus FJ, Bennett SJ, Kim EE, DeLand FH, Zahn MC, Goldenberg DM. Circulating immune complexes in cancer patients receiving goat radiolocalizing antibodies to carcinoembryonic antigen. Cancer Res 40:497–501, 1980.
13. Izzo MJ, Bale WF. Preferential localization and rate of loss of labeled alloantibody from rat tumors and skin transplants carrying the corresponding alloantigen. Cancer Res 36:2868–2873, 1976.
14. New and views. Nature (London) 248:637, 1974.
15. Sparks FC, Ting CC, Hammond WG, Herberman RB. An isotopic antiglobulin technique for measuring antibodies to cell-surface antigens. J Immunol 102:842–847, 1969.
16. Bale WF, Helmkamp RW, Davis TP, Izzo MJ, Goodland RL, Contreras MA, Spar IL. High specific activity labeling of protein with I^{131} by the iodine monochloride method. Proc Soc Exp Biol Med 122:407–414, 1966.
17. Ehrlich R, Witz IP. The elution of antibodies from viable murine tumor cells. J Immunol Methods 26:345–353, 1979.
18. Bale WF, Contreras MA, Izzo MJ, Della Penta D, Buchsbaum DJ. Preferential *in vivo* localization of ^{125}I-labeled antibody in a carcinogen-induced syngeneic rat tumor. Prog Exp Tumor Res 19:270–283, 1974.
19. Ey PL, Prowse SJ, Jenkin CR. Isolation of pure IgG_1, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A-sepharose. Immunochemistry 15:429–436, 1978.
20. Witz I, Klein G, Pressman D. Moloney lymphoma antibodies from mice; localization in spleens of

Moloney lymphoma bearing mice. Proc Soc Exp Biol Med 130:1102–1105, 1969.
21. De Vaux Saint Cyr Ch. Modulation of expression of virus-induced tumor antigens *in vivo* and *in vitro*. Isr J Med Sci 10:826–835, 1974.
22. Witz IP, Kinamon S, Ran M, Klein G. Tumour-bound immunoglobulins. The *in vitro* fixation of radioiodine-labelled anti-immunoglobulin reagents by tumour cells. Clin Exp Immunol 16:321–333, 1974.
23. Ran M, Klein G, Witz IP. Tumor-bound immunoglobulins. Evidence for the *in vivo* coating of tumor cells by potentially cytotoxic anti-tumor antibodies. Int J Cancer 17:90–97, 1976.
24. Izzo MJ, Contreras MA, Bale WF. Localization in rat skin transplants of purified ^{125}I-labeled xenogeneic histocompatibility antibody. Proc Soc Exp Biol Med 154:184–187, 1977.
25. Law MP, Thomlinson RH. Vascular permeability in the ears of rats after X-irradiation. Brit J Radiol 51:895–904, 1978.
26. Levine G, Ballou B, Reiland J, Solter D, Gumerman L, Hakala T. Localization of I-131-labeled tumor-specific monoclonal antibody in the tumor-bearing BALB/c mouse. J Nuclear Med 21:570–573, 1980.

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Immunocytochemical Localization of Catechol-*O*-Methyltransferase in Rat Parotid Gland (41478)

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Abstract. Catechol-*O*-methyltransferase (COMT) (EC 2.1.1.6) was localized in rat parotid gland using immunocytochemical methods. Immunoreactive deposits were found in the cytoplasm of myoepithelial cells, striated duct cells, myoepithelial-like cells of the small excretory duct, and small basal cells of the large excretory duct. The results confirm that the predominant localization of COMT is extraneuronal. After ligation of the main excretory duct, a marked reduction of COMT immunoreactivity was demonstrated in the striated duct. Chronic postganglionic sympathectomy did not produce a diminution of the COMT immunoreactivity in the parotid gland. The pattern of localization observed in the extraneuronal elements suggests that enzyme may function in extraneuronal inactivation of catechols in the parotid gland.

Catechol-*O*-methyltransferase (COMT) (EC 2.1.1.6) catalyzes the transfer of a methyl group from *S*-adenosylmethionine to one of the phenolic hydroxyls of a variety of catechols (1). This *O*-methylation reaction is important in the enzymatic inactivation of circulating catecholamines (2), the enzymatic inactivation of 2- and 4-hydroxyestrogens (3), the detoxification of xenobiotic catechols (4), and the local inactivation of catecholamines released as transmitters from the terminals of both central and peripheral catecholamine-containing neurons (5, 6).

Several biochemical studies have demonstrated the presence of COMT in salivary glands (7–9), and indirect evidence has suggested that COMT is localized both in extraneuronal and intraneuronal locations in the salivary gland (7, 10).

The localization of COMT in acinar and intercalated duct myoepithelial cells of the rat parotid gland has already been reported using immunocytochemical methods (11). In the present study we have shown, using the peroxidase-antiperoxidase method (12), that COMT is also present in striated duct cells, myoepithelial cells of the small excretory ducts, and in small basal cells of the excretory ducts. We have also examined the effects of sympathectomy and ligation of the excretory duct on the localization of COMT in the rat parotid gland.

Materials and Methods. Adult Wistar rats (200–400 g) of both sexes were used. Rats were anesthetized with Nembutal and perfused through the heart with 100 ml of periodate–lysine–paraformaldehyde (PLP) (13) 14 days after the surgical procedures. After perfusion the parotid glands were rapidly removed, cut into small pieces, and fixed with PLP for 3 hr at 4°. Tissues were dehydrated through graded alcohols and embedded in paraffin. Sections 8 μm in thickness were cut, mounted on glass slides with egg albumin and glycerine, and dried overnight at 37°. For immunocytochemical staining paraffin sections were deparaffinized with xylene and brought to water through graded alcohols.

Slides were washed with 0.1 *M* phosphate-buffered saline (PBS) and then treated with 0.005 *M* unbuffered periodic acid for 5 min and incubated with a solution of

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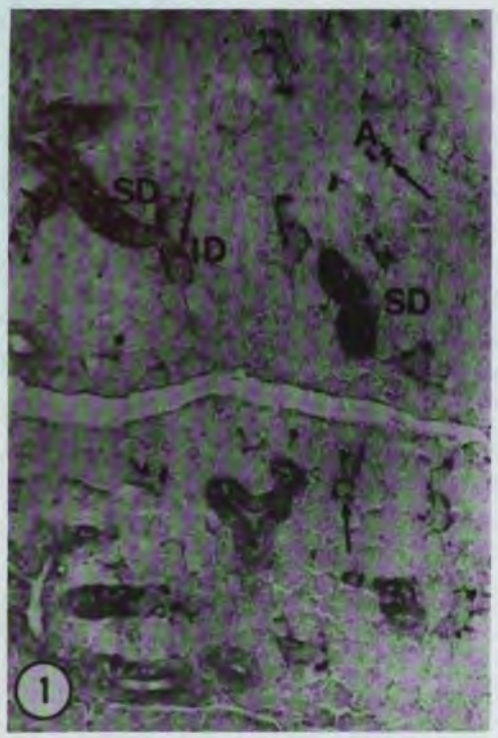
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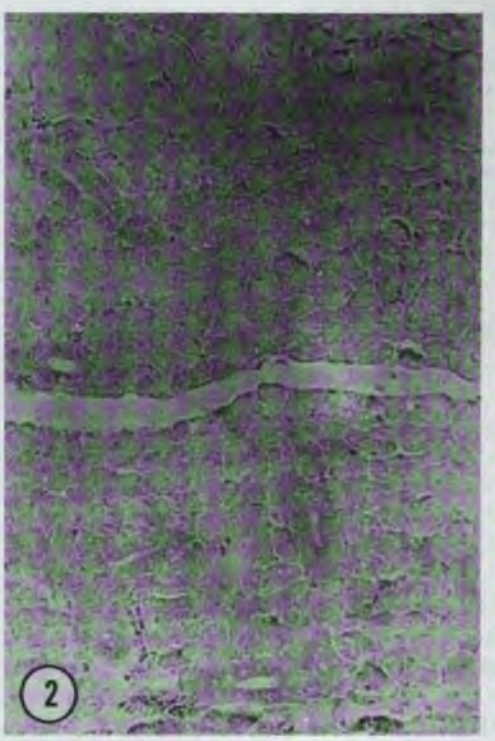
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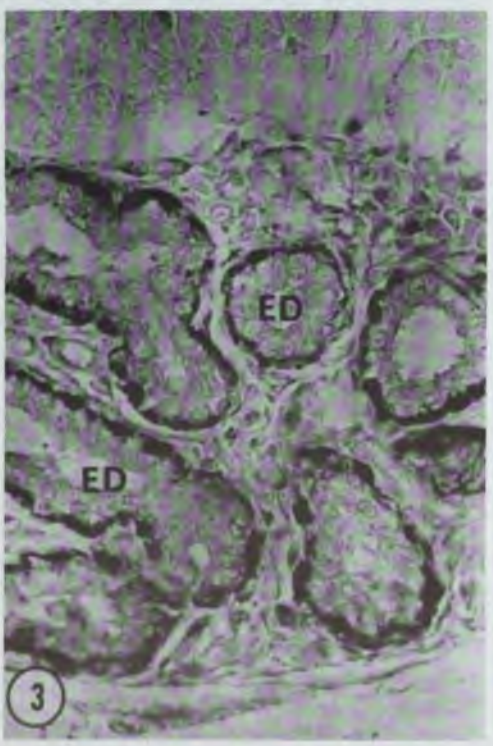
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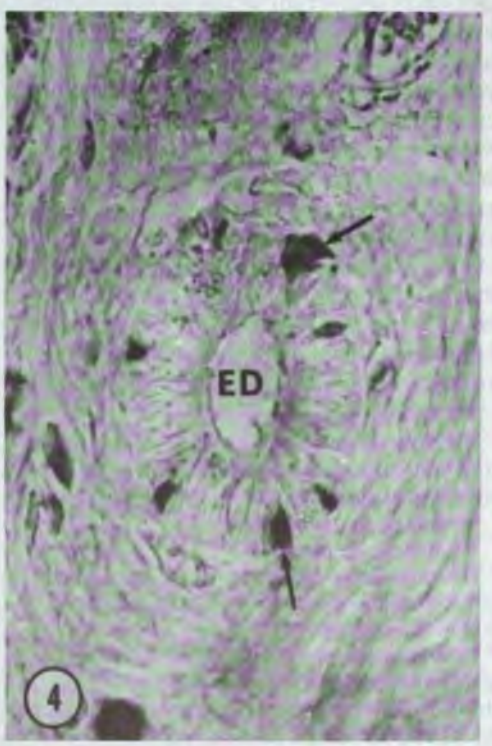
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$NaBH_4$ (10 mg/ml PBS) to inhibit endogeneous peroxidase activity (14). Sections were rinsed with two changes of PBS for 10 min and then incubated with a 1:50 dilution (in PBS) of normal goat serum for 30 min at room temperature.

Slides were incubated for 24 hr at 4° in a 1:1000 dilution (in PBS) of either rabbit antiserum to rat liver COMT (11), or as a control, in normal rabbit serum similarly diluted, a 1:10 dilution (in PBS) of goat anti-rabbit serum (Polyscience Inc., Warrington, Pa.) for 30 min at room temperature and then in a 1:10 (in PBS) dilution of peroxidase–antiperoxidase complex (PAP) (Polyscience Inc., Warrington, Pa.) for 45 min at room temperature. All slides were washed for 15 min each in three changes of PBS between each step. Peroxidase reaction product was developed by incubation of sections for 5 min in 3,3′-diaminobenzidine (0.05%) in 0.05 *M* Tris buffer, pH 7.6, containing 0.01% H_2O_2. After a final wash in distilled water the sections were dehydrated through graded alcohols, cleared with xylene, and mounted in Canada Balsam.

Atrophy of the parotid gland was produced by ligation of the excretory duct. The parotid glands were postganglionically sympathectomized by excision of the superior cervical ganglion. The operations were performed under Nembutal anesthesia. In all cases, the operation was unilateral, the intact side serving as a control.

Results. *Immunocytochemical reaction of COMT of the normal parotid gland (Figs. 1–4).* The sites of positive histological reaction product indicate the localization of interaction of the specific antiserum with tissue COMT and will be referred to as a COMT-positive reaction. COMT-positive product was observed in the cytoplasm of the myoepithelial cells of the acini and intercalated ducts, myoepithelial-like cells of the small excretory duct, and small basal cells of the large excretory duct. COMT was also present in the striated duct cells. Here, however, it appeared to be associated with basal striations. The differences in distribution were dramatically shown where transitions between striated and intercalated ducts were present in the sections (Fig. 1).

In the intralobular ducts the density of the COMT-positive reaction appeared much greater in the striated duct nearest the intercalated duct. The myoepithelial cells containing COMT-positive product were located at the bases of the acinar serous cells and intercalated duct (Fig. 1). Control sections of the parotid gland were negative (Fig. 2).

COMT-positive reaction was also present in myoepithelial-like cells located at the bases of small excretory ducts in the interlobular connective tissue (Fig. 3). Small basal cells containing COMT were found in the epithelium of the large excretory ducts (Fig. 4).

Immunocytochemical reaction of COMT following the surgical procedure (Figs. 5–9). 1. Superior cervical ganglionectomy (Figs. 5–7). Fourteen days after this procedure no histological changes were observed. COMT-positive product was present in the cytoplasm of the striated duct cells, myoepithelial cells (Figs. 5, 6), and small basal cells of the large excretory duct (Fig. 7). This distribution was similar to that of the normal parotid gland.

2. Ligation (Fig. 8). Ligation of the main excretory duct caused the expected histological changes in the parotid gland (see Refs. (10, 15)). The acinar cells not only

FIG. 1. Normal rat parotid gland. Strong specific staining for COMT is seen in the cytoplasm of the myoepithelial cells (arrows) and striated duct cells (SD) near the intercalated duct. A, acini; ID, intercalated duct. ×145.

FIG. 2. Normal rat parotid gland treated with normal rabbit serum as control. No staining for COMT is seen. ×145.

FIG. 3. Normal rat parotid gland. COMT is present in the myoepithelial-like cells of the small excretory duct (ED). ×410.

FIG. 4. Normal rat parotid gland. Small basal cells (arrows) of the large excretory duct (ED) contain COMT. ×655.


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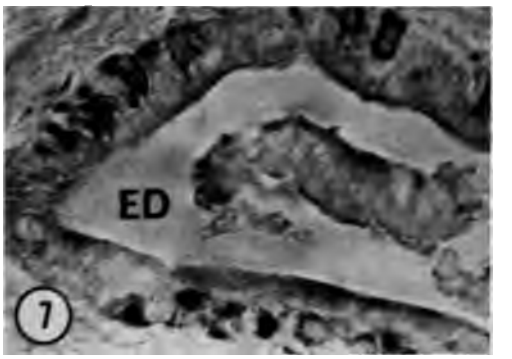
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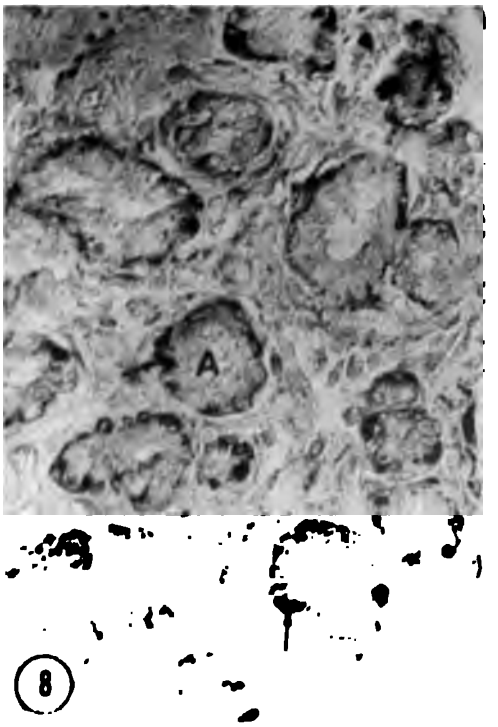
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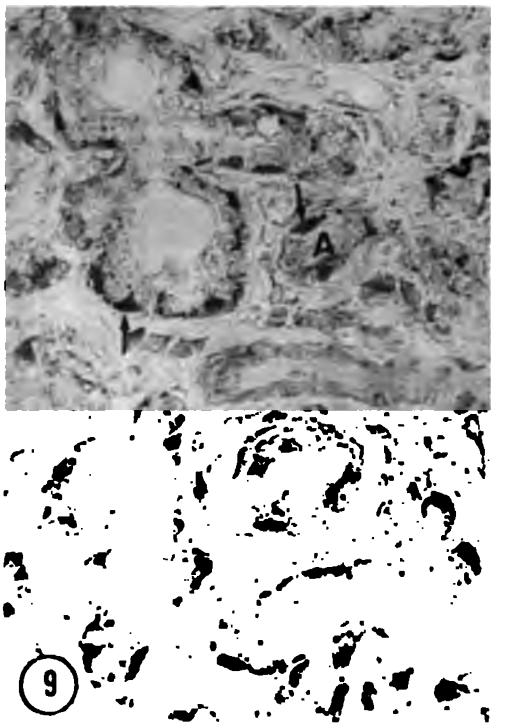
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atrophied, but also decreased in number. The intralobular and interlobular ducts had atrophied and their lumina were markedly dilated and the interstitial connective tissue had proliferated. The myoepithelial cells were intact and had COMT-positive reaction products (Fig. 8).

3. Ligation and postganglionic sympathectomy (Fig. 9). The glands that had been ligated and sympathectomized were similar to that seen after ligation alone. COMT was observed only in the myoepithelial cells (Fig. 9).

Discussion. In classical experimental approaches, salivary gland functions are differentiated by examination of the effects of ligation of the major excretory ducts or denervation of the gland. Following ligation the major secretory and ductal components of the gland, with the exception of the myoepithelial cells, undergo a striking atrophy (15, 16). After surgical, chemical, or immunological sympathectomy those functions of the gland dependent upon sympathetic innervation are lost (17, 18).

Previous studies have shown that rodent salivary glands contain both catecholamines and the enzymes, monoamine oxidase and COMT, responsible for their metabolic inactivation (17–20). Following sympathectomy, factors specifically related to intact sympathetic innervation such as the intraneuronal biosynthetic enzymes, tyrosine hydroxylase, and dopamine-β-hydroxylase, the tissue level of norepinephrine, and the high-affinity uptake system for norepinephrine all decline in parallel (18, 20). After atrophy due to duct ligation these factors remain essentially unchanged after appropriate adjustments are made for the reduction in glandular mass (10, 20).

Sympathectomy results in minimal reductions in monoamine oxidase levels while duct ligation leads to a profound decrease in enzyme levels. While it has been clearly established that monoamine oxidase is present in sympathetic neurons it is also widely distributed in other cell types in the salivary gland. Thus the minimal reduction in activity following degeneration of sympathetic neurons is consistent with the distribution of the enzyme. In the case of COMT, sympathectomy results in very minor changes in enzyme activity in the parotid gland (15). Similar studies in the submaxillary gland of rat indicate a sympathectomy-dependent decrease in COMT activity and suggest the presence of intraneuronal COMT (8).

However, duct ligation results in a partial and quite variable reduction of COMT activity ranging from 10 to 40% of the activity in unligated glands. A major fraction of COMT activity is unaffected in the presence of a major atrophy of the gland (8, 15, 20).

Our present results provide direct, visual confirmation of these conclusions. As in other tissues studied (11, 21, 22), we have found no evidence for a neuronal localization for COMT. In the salivary gland, COMT was found in the striated duct cells, the small basal cells of the excretory ducts, and in myoepithelial cells. In those locations the enzyme is not detectably affected by sympathectomy. Ductal ligation led to a variable loss in COMT located in striated duct cells and no detectable change in COMT in myoepithelial cells. The myoepithelial localization of COMT was unchanged following both duct ligation and sympathectomy. Thus the variable de-

FIG. 5. Rat parotid gland after removal of the superior cervical ganglion. COMT reaction products are seen in the cytoplasm of the myoepithelial cells (arrows) and striated duct cells (SD). A, acini; IR, intercalated duct. ×190.

FIG. 6. Rat parotid gland after removal of the superior cervical ganglion. Specific staining for COMT is observed in the myoepithelial-like cells of the small excretory duct (ED). ×425.

FIG. 7. Rat parotid gland after removal of the superior cervical ganglion. COMT reaction product are found in the small basal cells of the large excretory duct (ED). ×535.

FIG. 8. Rat parotid gland following ligation of the main excretory duct. The myoepithelial cells (arrows) contain COMT. A, acini. ×410.

FIG. 9. Rat parotid gland following ligation of the excretory duct and removal of the superior cervical ganglion. The myoepithelial cells (arrows) contain COMT. A, acini. ×400.

crease in COMT activity following duct ligations may reflect the loss of striated duct cells and possibly the small basal cells of the excretory duct. The major fraction of COMT activity which is unaffected by ligation-induced activity clearly appears to be associated with myoepithelial cells.

The precise function of COMT in these extraneuronal sites is problematic. There is little information about the functional role of basal cells in the salivary gland excretory ducts. A similar question can be raised concerning the role of COMT in the small, basal cells of the rat epididymis (11). With regard to myoepithelial cells on the other hand, conventional thinking suggests that the presence of COMT in contractile myoepithelial cells is related to the inactivation of norepinephrine released locally from noradrenergic varicosities or perhaps by direct sympathetic innervation of myoepithelial cells. Thus, by analogy with cholinergic nerve–muscle relationships, COMT has been presumed to be the noradrenergic analog of acetylcholinesterase.

Certain aspects of this analogy are of interest. The contractile function of myoepithelial cells, first suggested as early as 1661 (23), is well documented (24). These cells contain filaments similar to those of smooth muscle cells; they respond to sympathetic stimulation by contraction leading to increased ductal pressure. The response is mediated through an α-adrenergic receptor and has been observed directly in organ culture. Further, it is known that the acini of rat parotid glands are closely associated with catecholaminergic, fluorescent varicosities. It is of interest to note that a fraction of norepinephrine uptake by salivary gland is reserpine resistant, suggesting a significant participation of extraneuronal uptake sites for norepinephrine. Furthermore, reserpine treatment results in a partial loss of COMT activity within 6–18 hr (18). These findings suggest that both norepinephrine uptake and the reserpine-induced reduction of COMT activity may occur in myoepithelial cells. The possibility that the reserpine-sensitive fraction of COMT in salivary glands is present in myoepithelial cells is under examination by our immunocytochemical procedure to determine whether this effect occurs in myoepithelial cells or elsewhere.

Recent experimental results have greatly weakened any direct functional or morphological analogy between acetylcholinesterase and COMT. Localization of COMT in brain by fluorescent immunocytochemical techniques have demonstrated glial and ependymal cell localization rather than a neuronal localization. Other major sites of COMT are the choroid plexus (22) and the ciliary body of the eye (25). It has been suggested that in these sites COMT functions as a barrier or "enzymatic sink" to the passage of free catechols which restrict neuronally released catechols to a local area (25). Studies of the localization of COMT in the reproductive tract suggest a similar function of the enzyme as a protective barrier or "enzyme-sink" for catecholestrogens (26). Finally it should be noted that COMT has been demonstrated immunocytochemically in the ductal cells of the lactating rat mammary gland where it may play the same role as in the salivary gland (27, 28). Thus it is clear that further investigations will be required to define the nature of the role of COMT in salivary gland myoepithelial cells with certainty.

We wish to thank Mrs. Cahill, Mr. Carter, and Mr. Nakamura for their skilled technical assistance, and Ms. Colleen LePore for her clerical assistance.

1. Axelrod J. Methylation reactions in the formation and metabolism of catecholamines and other biogenic amines. Pharmacol Rev 18:95–113, 1966.
2. Kopin J, Axelrod J, Gordon E. The metabolic fate of H^3-epinephrine and C^{14}-metanephrine in the rat. J Biol Chem 236:2109–2113, 1961.
3. Merriam GR, MacLusky NJ, Johnson LA, Naftolin F. 2-Hydroxyestradiol-17α and 4-hydroxyestradiol-17α, catecholestrogen analogs with reduced estrogen receptor affinity. Steroids 36:13–20, 1980.
4. Creveling CR, Dalgard N, Shimizu H, Daly JW. Catechol-O-methyltransferase III: Meta- and para-O-methylation of catechols. Mol Pharmacol 6:691–696, 1970.

5. Guldberg HC, Marsden CA. Catechol-O-methyltransferase: Pharmacological aspects and physiological role. Pharmacol Rev 27:135–206, 1975.
6. Jarrott B. Occurrence and properties of catechol-O-methyltransferase in adrenergic neurons. J Neurochem 18:17–27, 1971.
7. Iversen LL, Glowinski J, Axelrod J. The physiologic disposition and metabolism of norepinephrine in immunosympathectomized animals. J Pharmacol Exp Ther 151:273–284, 1966.
8. Broch OJ. Effect of reserpine on catechol-O-methyltransferase in rat submaxillary gland. J Pharm Pharmacol 26:375–377, 1974.
9. Ikeno T, Ikeno K, Hashimoto S, Nemoto K, Hasegawa J, Kuzuya H. Catecholamine metabolism in salivary glands of the rats. 3. Developmental changes in catechol-O-methyltransferase activity. J Tohoku Dent Univ 4:82–85, 1977.
10. Anden N-E, Norberg K-A, Ison L. The adrenergic nerves of rat rat salivary glands after excretory duct ligation. Acta Physiol Scand 66:501–506, 1966.
11. Inoue K, Tice LW, Creveling CR. Immunocytochemical localization of catechol-O-methyltransferase. In: Usdin E, Weiner N, Youdim MBH, eds. Biochemistry and Function of Monoamine Enzymes. New York, Dekker, pp835–859, 1977.
12. Sternberger LA. Enzyme immunocytochemistry. In: Hayat MA, ed. Electron Microscopy of Enzymes. Principles and Methods. New York, Van Nostrand Reinhold, pp150–191, 1973.
13. McLean IW, Nakane PK. Periodate–lysine–paraformaldehyde fixative, a new fixative for immunoelectron microscopy. J Histochem Cytochem 22:1077–1083, 1974.
14. Isobe Y, Chen S-T, Nakane PK, Brown WR. Studies on translocation of immunoglobulins across intestinal epithelium I. Improvements in the peroxidase-labeled antibody method for application to study of human intestinal mucosa. Acta Histochem Cytochem 10:161–171, 1977.
15. Marsden CA, Broch OJ, Guldberg HC. Catechol-O-methyltransferase and monoamine oxidase activities in rat submaxillary gland: Effects of ligation, sympathectomy and some drugs. Eur J Pharmacol 15:335–342, 1971.
16. Garrett JR, Kemplay SK. The adrenergic innervation of the submandibular gland of the cat and the effects of various surgical denervations on these nerves. A histochemical and ultrastructural study including the use of 5-hydroxydopamine. J Anat 124:99–115, 1977.
17. Haggendal J. The disappearance of dopamine-β-hydroxylase from rat salivary gland after extirpation of the superior cervical ganglion. J Neural Transmission 48:249–259, 1980.
18. Kuzuya H, Ikeno T, Ikeno K, Memoto K, Hashimoto S. Catecholamine contents and activities of catecholamine synthesizing and inactivating enzymes in the salivary glands of young growing rats. Arch Oral Biol 25:31–36, 1980.
19. Almgren O, Anden N-E, Jonason J, Norberg K-A, Olson L. Cellular localization of monoamine oxidase in rat salivary glands. Acta Physiol Scand 67:21–26, 1966.
20. Jonason J. Metabolism of dopamine and noradrenaline in normal, atrophied and postganglionically sympathectomized rat salivary glands in vitro. Acta Physiol Scand 76:299–311, 1969.
21. Kaplan GP, Hartman BK, Creveling CR. Immunohistochemical demonstration of catechol-O-methyltransferase in mammalian brain. Brain Res 167:241–250, 1979.
22. Kaplan GP, Hartman BK, Creveling CR. Anti-catechol-O-methyltransferase: Demonstration of specificity and immunological cross reactivity with the enzyme from rat liver, kidney, brain and choroid plexuses. Neurochem Res 5:869–877, 1980.
23. Stenonis N. Glandulis oris et novis earundem vasis, 1661. Bodlei Library, Oxford.
24. Garrett JR, Emmelin N. Activities of salivary myoepithelial cells: A review. Med Biol 57:1–28, 1979.
25. Creveling CR, Hartman BK. Relationships between the cellular localization and the physiological function of catechol-O-methyltransferase. In: Borchardt RT, Creveling CR, Usdin E, eds. Proceedings of the Conference on Transmethylation, Lake of the Ozarks, October 26–29, 1981. London, Macmillan, pp479–486, 1982.
26. Inoue K, Tice LW, Creveling CR. Immunocytochemical localization of catechol-O-methyltransferase in the pregnant rat uterus. Endocrinology 107:1833–1840, 1980.
27. Lowe MC, Amin AF, Creveling CR. Immunocytochemical localization of catechol-O-methyltransferase in normal and cancerous breast tissues. In: Borchardt RT, Creveling CR, Usdin E, eds. Proceedings of the Conference on Transmethylation, Lake of the Ozarks, October 26–29, 1981. London, Macmillan, pp487–490, 1982.
28. Strum JM. Estrogen-induced alterations in the myoepithelial cells of the rat mammary gland. Cell Tissue Res 193:155–161, 1978.

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Light and Electron Microscopic Studies of the Pathogenesis of Vaccinia Virus Infection in Mouse Brain (41479)

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Abstract. Vaccinia virus replication in weanling mouse brains was examined by light and electron microscopy 2 to 5 days after inoculation, i.e., during the period of maximal viral infectivity. Replication sites were detected in meningeal cells, adventitial cells of meningeal arterioles, and small nonneuronal cells of the brain. No evidence was found for replication in neurons, although these cells were altered indirectly. The virus can be described most accurately as being leptomeningoencephalitic.

The natural history of poxvirus infection usually includes involvement of the respiratory tract, reticuloendothelial system, liver, and skin. Infection of the brain is rare, but is of interest because of the possibility that encephalitis following immunization with vaccinia virus may be a manifestation of infection. It is unlikely that it results from a toxic effect (1). Experimental inoculation of brain with vaccinia virus has revealed that some strains proliferate in nervous tissue. Uncertainties exist, however, as to the types of cells involved and the natural history of intracerebral infection. Several reports have appeared suggesting that the principal lesions of cerebral infections are a leptomeningitis and/or ependymitis (2–5). Most of these studies have been conducted in mice, and the approach has been principally through light microscopy, including the application of immunofluorescence. An exception to the usual reports has been a study in which a racoon poxvirus inoculated into mice was found to cause necrotic foci in dorsal spinal ganglia and severe demyelination with necrosis in the lumbar plexuses (6). These findings suggest a modification of neurons due to a direct viral infection. Where it has been considered, there is no evidence for viral replication in glial cells. Immunofluorescence microscopy is not sensitive enough to justify the conclusion that immunofluorescent-negative cells are not infected. Conversely, positive immunofluorescence does not guarantee intracellular viral replication. Electron microscopy permits direct observation of the characteristic morphology of poxviruses in both their replicating and mature forms. Accordingly, electron microscopy was brought to bear on the question of poxvirus neurovirulence and focused on the early period of maximal infectivity after the intracerebral inoculation of mice with vaccinia virus.

Materials and Methods. *Poxvirus.* Vaccinia virus, strain Levaditi-58 (7), is a virus capable of multiplying to a high level in mouse brain. It was assayed by dropping the virus on the chorioallantois of 11-day old chicken embryos, and after 2 days of incubation at 37° the results were scored as pock-producing units (ppu) per 0.05 ml of inoculum.

Mice. Three-week-old, NIH general purpose albino mice were employed. Virus was administered by injecting 0.02 ml of suspension into the right cerebral hemisphere of ether-anesthetized mice.

Viral replication in brain. Eighteen mice were inoculated with a selected virus dose and three mice were sacrificed each day thereafter, at which time the pooled brains were ground with RR Alundum and brought to a 20% suspension, by weight, in 0.85% NaCl solution. From the growth curve results, a suitable virus dose (100 ppu) was selected for further studies. Control mice, sham inoculated with NaCl solution, were run in parallel.

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Preliminary preparation of infected brain. For 6 days after intracerebral inoculation with 100 ppu of virus, each of two mice was anesthetized, and an infusion was begun through the left ventricle of the heart with 20 ml of cold (4°) imidazole–formalin solution (0.1 *M* imidazole–HCl, 10% formalin, pH 7.2). As the infusion was begun, the hepatic vein was cut. After infusion, the brain and spinal cord were dissected out and stored in imidazole–formalin solution at 4° for at least 24 hr. Control mice were processed in an identical manner.

Fixation and sectioning of brain. The cold, fixed brain was cut coronally into eight serial sections. Subsections for light microscopy were embedded in Paraplast and the caudal surface was sectioned to a thickness of 5 μm. The sections were stained with hematoxylin and eosin.

The specimens for electron microscopy were divided further by a medial cut, dividing them into right and left sides of the brain. Prior to postfixation, further subsections were made. The subsections were diced into pieces less than 1 mm in greatest diameter and were fixed in isoosmolal, buffered, 1.5% glutaraldehyde, pH 7.4, for 3 hr at 4° and then placed in a wash solution (isoosmolal sucrose solution with 0.02 *M* imidazole–HCl, pH 7.4) overnight at 4°. (The tissue used for electron microscopy had been kept at 4° until this point.) After a 1-hr exposure to 2% OsO_4 in sodium barbital buffer at pH 7.4, the specimens were dehydrated through an alcohol series, carried in propylene for two 15-min periods, held for 30 min in a 50/50 propylene–Maraglas mixture and embedded in Maraglas as described previously (1). Thin sections (about 500 nm) were cut with glass knives, and areas to be studied by electron microscopy were selected. Ultrathin sections (about 75 nm) were cut with diamond knives and placed on uncoated copper grids, stained with lead citrate, and studied by electron microscopy (1).

Results. *Viral replication in brain.* Vaccinia virus replication at three different doses is shown in Fig. 1. The virus replicates appreciably by 3 days in mouse brain. Accordingly, for electron microscopic studies the combination of a dose of 100 ppu and an examination period of 2–4 days postinoculation was selected as conditions under which viral replication would be expected to be extensive and yet tissue breakdown would be at a minimum.

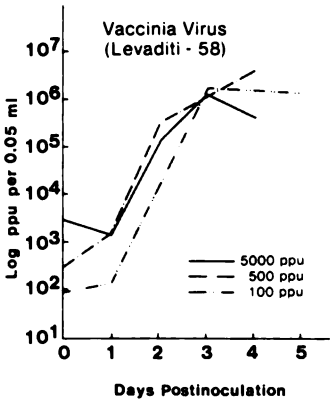

FIG. 1. Replication of vaccinia virus in mouse brain following intracerebral inoculation with three different doses of virus.

Signs of disease. At the 100 ppu dose, signs of disease began to appear at 3 days postinoculation. The mice appeared lethargic, hunched and trembling. Flaccid paralysis was noted, and body spasms occurred when the mice were twirled by their tails. By the fourth day, trembling continued and breathing became labored. The front legs no longer were used for locomotion. Animals surviving to the fifth day became totally immobilized, very sensitive to noise vibrations, and usually died in spasms. At this time, the brains were of a very soft consistency and covered with petechial hemorrhages. Some mice survived to Day 6, but none survived longer.

Light microscopy of infected brains. Histologically, the brain was essentially unremarkable during the first 2 days postinoculation (Fig. 2A). By the third and fourth days, many cortical areas showed nuclear shrinkage associated with early, occasionally marked, perinuclear clearing, especially in the neuronal layers (Fig. 2B). More extensive perinuclear clearing was present by the fifth day. A focal leptomeningitis and ependymitis, both of which included a polymorphonuclear

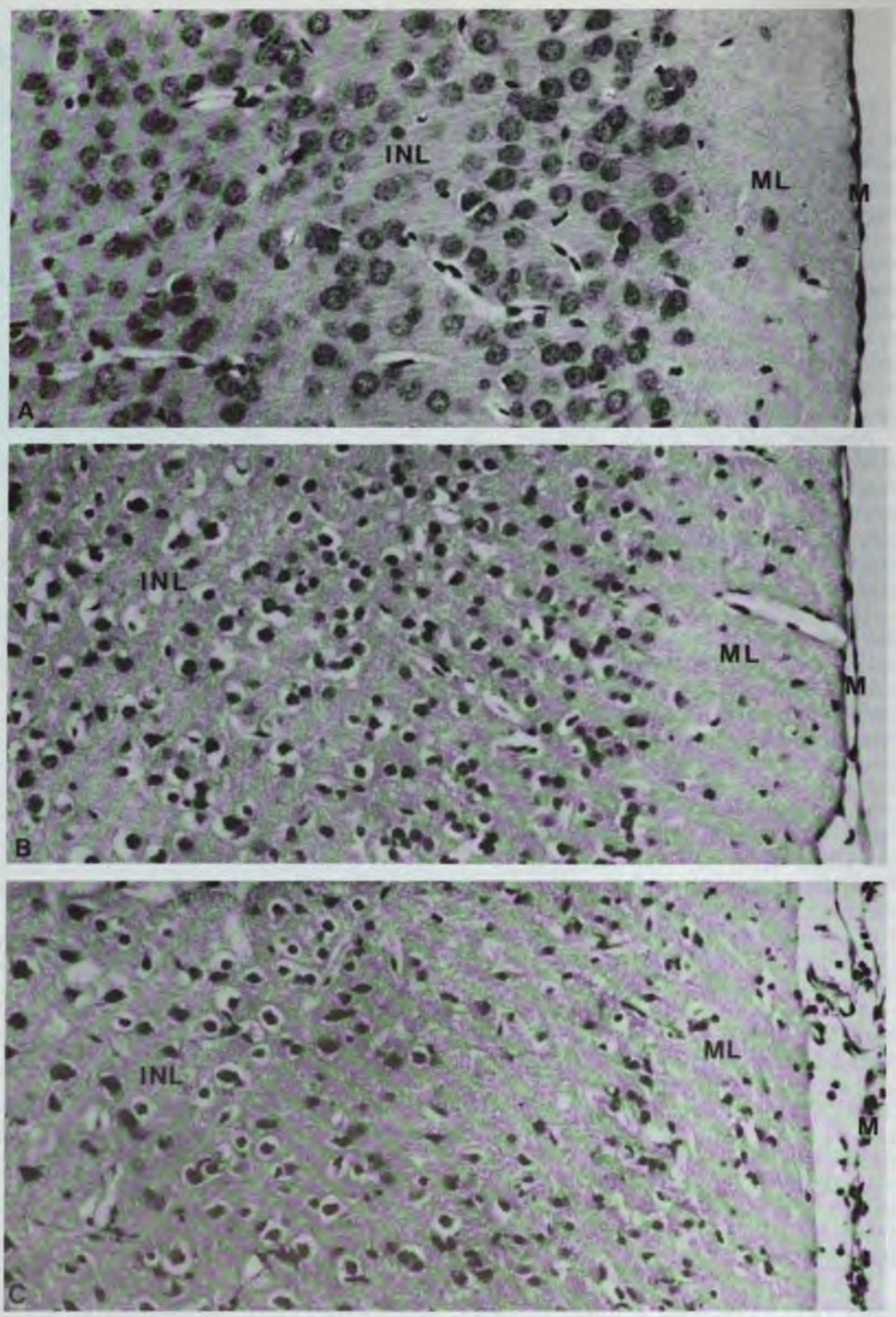

FIG. 2. Light microscopy of dorsal surface of parietal cortex of brain. (A) One day after virus inoculation. Meninges (M), molecular layer (ML), and inner neuronal layer (INL) appear normal. (B) Four days postinoculation. Nuclear shrinkage, nuclear hyperchromasia, and perinuclear clearing are evident in cells of the molecular and inner neuronal layers, especially cells that appear to be neurons. The neuropil has a spongy quality. Note that the meninges appear unremarkable. (C) Six days postinoculation. Changes in molecular and inner neuronal layers persist. In addition, an inflammatory infiltrate, rich in neutrophils, involves the meninges. Hematoxylin and eosin, ×250.

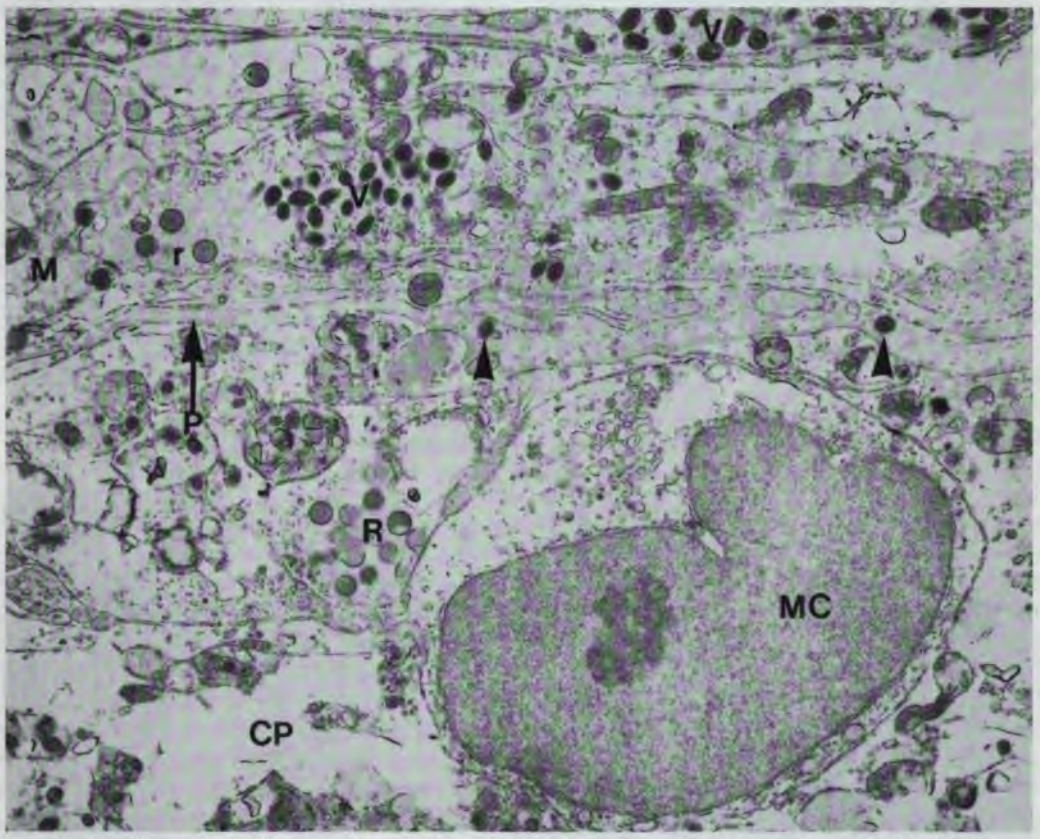

FIG. 3. Three days after virus inoculation. Meninges and submeningeal cortex. Above the pia (P), most meningeal cells (M) contain clusters of mature virus (V) and/or sites of viral replication (r). Arrowheads indicate individual mature viruses within the pia. Below the pia, the molecular layer neuropil contains swollen cytoplasmic processes (CP), one of which contains a site of viral replication (R). A mononuclear cell (MC) shows cytoplasmic swelling. Lead citrate, ×6600.

leukocyte infiltrate, developed between the fourth and sixth days. Generally, the focal leptomeningitis first appeared on the ventral surface and later (at 5–6 days) on the dorsal surface (Fig. 2C). Diffuse leptomeningitis and focal ependymitis found in terminal mice were occasionally associated with focal necrosis of the underlying brain.

Electron microscopy of infected brains. Brain sections from sham-inoculated control mice were well preserved and unremarkable in appearance.

In inoculated animals, virus was not readily demonstrable at 2 days. Mature virus and sites of viral replication were readily found at 3–4 days, at which time considerable degenerative alterations were noted in meningeal, neuronal, glial, ependymal, and vascular adventitial cells. In contrast, endothelial and smooth muscle cells of the cerebral vasculature were preserved. Viral structures were most easily found at 3 days, which corresponds with the peak of infectious virus titer (Fig. 1).

Between 3 and 4 days, both mature virus and sites of viral replication were clearly identified in many cells; meningeal cells were extensively involved (Figs. 3, 4) and adventitial cells of meningeal arterioles were also commonly involved (Fig. 4). In the cerebral cortex and cerebellum, numerous small, mononuclear cells of the neuropil of the molecular and neuronal layers contained sites of viral replication and mature virus (Fig. 5). The virus-containing cells frequently were adjacent to capillaries and neurons. These cells were interpreted as probably being glial cells.

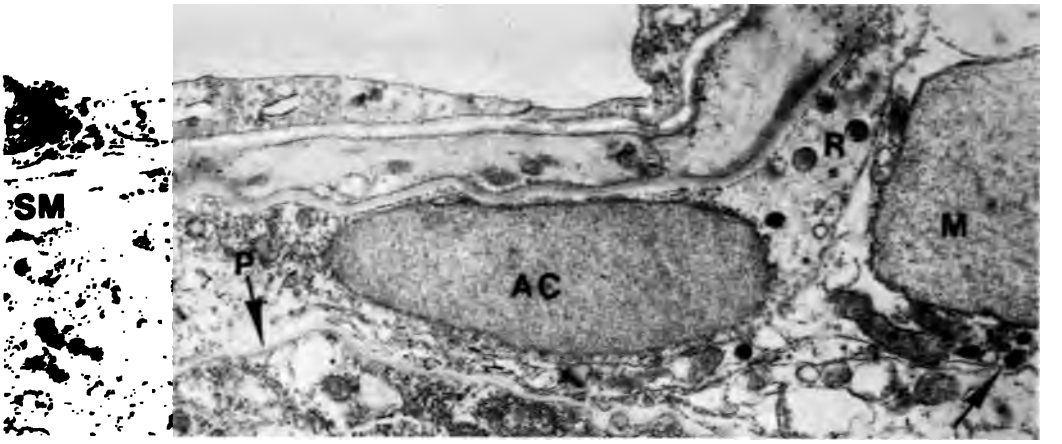

FIG. 4. Three days after virus inoculation. Section of meninges (above) and subjacent molecular layer. An arteriolar wall shows no virus in the capillary endothelial cells (En) or smooth muscle cells (SM). An adventitial cell (AC) contains a site of viral replication (R). Mature virus (arrow) can be seen in the cytoplasm of an adjacent meningeal cell (M). Pia (P). Lead citrate. ×6400.

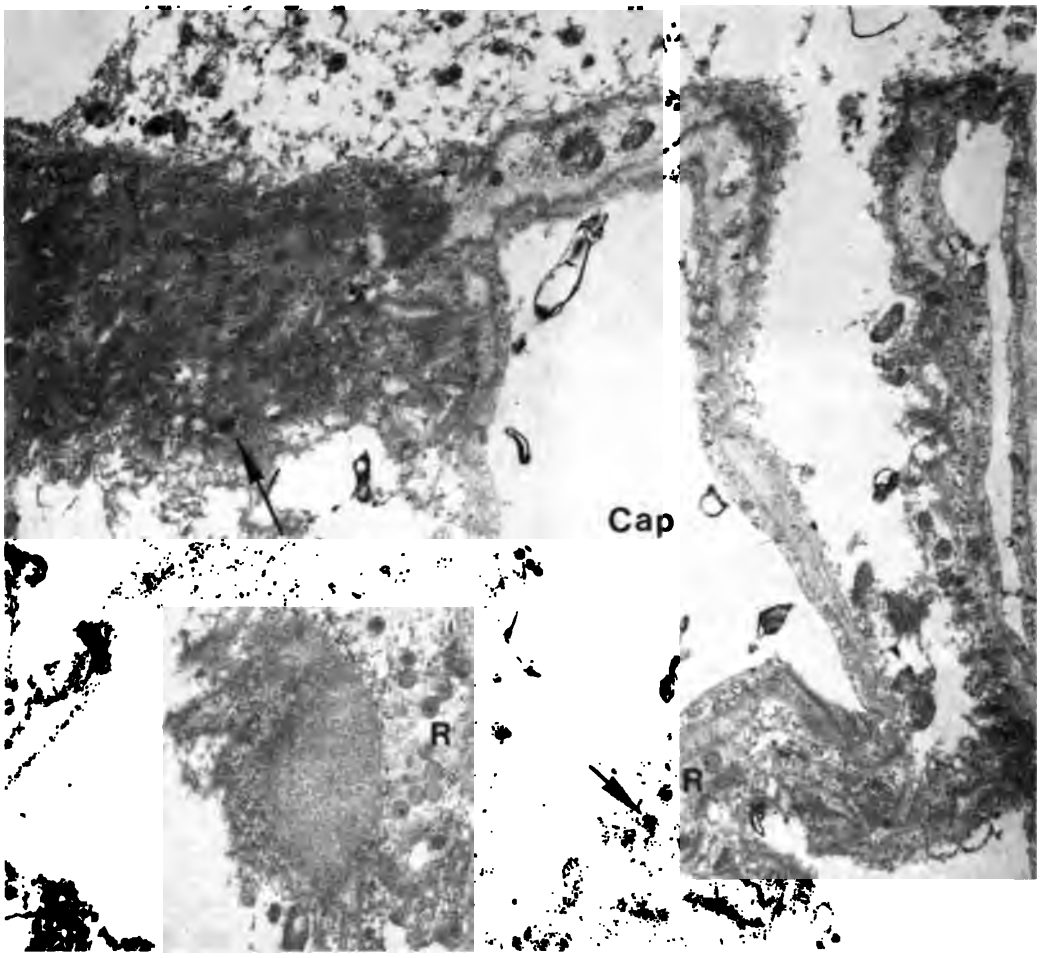

FIG. 5. Three days after virus inoculation. Cerebral cortex. A mature virus particle (short arrow) and sites of viral replication (R) are seen in two small paracapillary cells showing marked cytoplasmic swelling. A cell with dense cytoplasm contains a mature virus particle (long arrow). Capillary lumen (Cap). Lead citrate. ×6200.

To determine whether neurons contained viral material, a number of sections of cerebral cortex rich in neurons, as determined by light microscopy, were carefully selected and studied by electron microscopy. In no instance were viral structures found in neuronal cells, which frequently showed cytoplasmic clearing and other degenerative changes. In addition, viral structures never were identified in ependymal cells, endothelial cells, smooth muscle cells of blood vessels, or cells of the inflammatory infiltrates.

Discussion. Previous investigators have shown the presence of viral antigen in leptomeningeal cells of vaccinia virus-infected mouse brains, using immunofluorescence techniques (2, 4). The present study confirms that the meninges are a principal site of intracranial replication of vaccinia virus during the first 4 days after inoculation of brain and for the first time demonstrates unequivocally the presence of replicating viral structures in meningeal cells. In addition, virus replicates in the adventitial cells of blood vessels, principally meningeal arterioles, and in small nonneuronal cells of the cerebral cortex, presumably glial cells. Although ependymitis was observed, no virus was detected in ependymal cells, which frequently were focally denuded.

Blinzinger and co-workers, using electron microscopy, studied late stages of infection, i.e., 4–6 days postinoculation in adult mice (5). At that time, they found virus only in mononuclear phagocytes and arterial adventitial cells of the meninges. No evidence of neuronal, glial, or ependymal cell infection was found. The present study focuses on earlier stages of infection, in weanling mice, in contrast to Blinzinger's examination of the later stages of infection in adult mice. These differences in experimental design appear to account for the variation in findings. In fact, it is postulated from the results of these two studies that after initial infection of leptomeningeal, vascular adventitial, and glial cells of the brain, a secondary infection of late occurring inflammatory infiltrate may ensue.

In keeping with Blinzinger *et al.* (5), virus was not found within neurons, although in the present study marked alterations of these cells were noted by both light and electron microscopy. The neuronal changes are in parallel with a report on the indirect action of Newcastle disease virus on the neurons of mice (8). Since glial cells are thought to influence the transmission of nervous activity (9), consideration of this point may be appropriate in the present study. It has been shown that in primary cell cultures astrocytes release factors into the medium that promote the growth and prolong the survival of rat hippocampal neurons (10). In this regard, in the present study it was noted that small nonneuronal cells within the cerebral cortex frequently contained sites of viral replication. Although marked alterations of these cells, probably due to viral replication, precluded definitive structural identification, the cells presumably represent glial components. This presumption is supported by the absence of cellular inflammatory infiltrates, which might lead to the confusion of cell types within the brain, during the time period covered in this study.

The term "neurotropic" has been applied to numerous viruses replicating in the brain. It has been suggested that this is an unsuitable designation for poxviruses replicating in mouse brain since they do not replicate in neurons (11). Modern dictionaries, however, define "neurotropic" as designating agents having an affinity for nervous tissue (12, 13), thus making the term applicable to the aforementioned poxviruses. In the present study, viral replication (or infection) within neurons could not be detected, even though virus replicated in nonneuronal cells of the brain substance. The virus, however, effected an indirect neuronovirulence, as suggested by the observed paralysis of infected animals and the morphologic alterations in neurons. Care should be taken to recognize that some viruses are strictly "neuronotropic," replicating only within neurons. Viruses replicating in the brain also may be described as "encephalitic," causing inflammation of the brain, as distinct from its membranes (14). The virus examined in this study can be most specifically described as being leptomeningoencephalitic.

The technical assistance of Mr. Willy B. Thomas is gratefully acknowledged.

1. Sottnek HM, Campbell WG, Jr, Cassel WA. The pathogenesis of vaccinia virus toxicity. II. An electron microscopic study. Lab Invest 33:522–532, 1975.
2. Mimms CA. Intracerebral injections and the growth of viruses in the mouse brain. Brit J Exp Pathol 41:52–59, 1960.
3. Soekawa M, Morita C, Moriguchi R, Nakamura M. Neurovirulence of vaccinia viruses in mice. Zentralbl Bakteriol Parasitenk Infectionskr Hyg, Abt I 226:434–442, 1974.
4. Ginsberg AH, Johnson KP. Vaccinia virus meningitis in mice after intracerebral inoculation. Infect Immun 13:1221–1227, 1976.
5. Blinzinger K, Hochstein-Mintzel V, Anzil AP. Experimental vaccinia virus meningoencephalitis in adult albino mice. Virological, light microscopic and ultrastructural studies. Acta Neuropathol 40:193–205, 1977.
6. Thomas EK, Palmer EL, Obijeski JF, Nakano JH. Further characterization of racoonpox virus. Arch Virol 49:217–227, 1975.
7. Cassel WA, Garrett RE. Relationship between viral neurotropism and oncolysis. I. Study of vaccinia virus. Cancer 20:433–439, 1967.
8. Burks JS, McFarland HF, Narayan O. A unique neurological disease of mice induced by a paramyxovirus, Newcastle disease virus. Neurology 25:350, 1975.
9. Glees P. Neuroglia: Morphology and Function. Springfield, Ill, Thomas, pp49–53, 1955.
10. Banker GA. Trophic interactions between astroglial cells and hippocampal neurons in culture. Science 209:809–810, 1980.
11. Fenner F, McAuslan BR, Mimms CA, Sambrook J, White DO. The Biology of Animal Viruses. New York, Academic Press, p375, 1974.
12. Stedman's Medical Dictionary, 23rd ed, Baltimore, Williams & Wilkins, 1976.
13. Dorland's Illustrated Medical Dictionary, 26th ed, Philadelphia, Saunders, 1981.
14. Longson M. The general nature of viral encephalitis in the United Kingdom. In: Illis LS, ed. Viral Diseases of the Central Nervous System. Baltimore, Williams & Wilkins, p19, 1975.

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The Yucatan Miniature Swine: An Improved Pig Model for the Study of Desoxycorticosterone-Acetate (DOCA) and Aldosterone Hypertension (41480)

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Abstract. Blood pressure responses to chronic desoxycorticosterone-acetate (DOCA) or *d*-aldosterone (Aldo) administration in the intact adult Yucatan miniature boar were investigated. Daily pressure measurements were made between 9:00 AM and noon from Tygon carotid artery catheters. Following several days of baseline observations, DOCA-impregnated silastic strips, Aldo-impregnated silastic strips, or silastic alone (control) were implanted subcutaneously in the right or left flank under light thiamylal (Surital) anesthesia. Observations were continued for 3 weeks. Blood pressures with either steroid were significantly different from control within one week postimplantation. They continued to rise throughout the study period from a preimplant level of 100–110 mg Hg to a mean of 140 mm Hg. These studies demonstrate that the Yucatan miniature boar, in contrast to other animal models previously described, will readily develop hypertension with either DOCA or Aldo and that such studies can be conducted in the intact adult animal.

It has previously been reported that young, uninephrectomized farm pigs respond to desoxycorticosterone-acetate (DOCA) implantation with a rapid and consistent rise in blood pressure (1, 2). While these animals responded more reliably than dogs and rats, the use of a rapidly growing, pediatric animal presented difficulty in the interpretation of data (1, 3). Since this model was really a characterization of juvenile hypertension, it was questioned whether the responses observed could be directly extrapolated to the adult hypertensive process. Adult domestic farm pigs can weigh 300 kg or more. Studies utilizing these animals in their adult stage were therefore not feasible. The present study was undertaken to determine whether the adult Yucatan miniature swine would be suitable for studies in this area. Mature Yucatan miniature boars have a mean weight of 83 ± 12 kg (range 59–105 kg) (4) and, when treated correctly, are extremely docile. These features of the breed permit measurements to be made in the unrestrained adult animal, and, since it has been shown that uninephrectomy is not required (5, 6), hypertension studies can be conducted in the intact animal. Additionally, it was of interest to determine whether the intact adult animal would respond to the administration of *d*-aldosterone (Aldo) as well as DOCA with an increase in blood pressure and whether the responses would be similar. Significant aldosterone hypertension in an intact adult animal model has not been previously reported.

Materials and Methods. Experimental animals (18–28 months old, 70–100 kg), were housed in 4 × 6-ft metabolic cages for observation periods of 2–4 weeks before undergoing surgical procedures. Throughout the study all animals received a premeasured quantity of pig chow meal (Zeigler Brothers, Gardners, Pa.) supplemented with sodium chloride to allow a controlled sodium intake of 4.5 meq/kg/day. Water was provided *ad libitum*. All metabolic measurements had stabilized by the end of the observation period.

An indwelling carotid artery catheter (Tygon 0.040-in. i.d., 0.070-in. o.d.) was then placed in each experimental animal. Following preanesthesia with ketamine (20

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Although Aldo has been used in a number of [illegible] with rats, the ability of this hormone to produce hypertension has been disappointing and the results have been

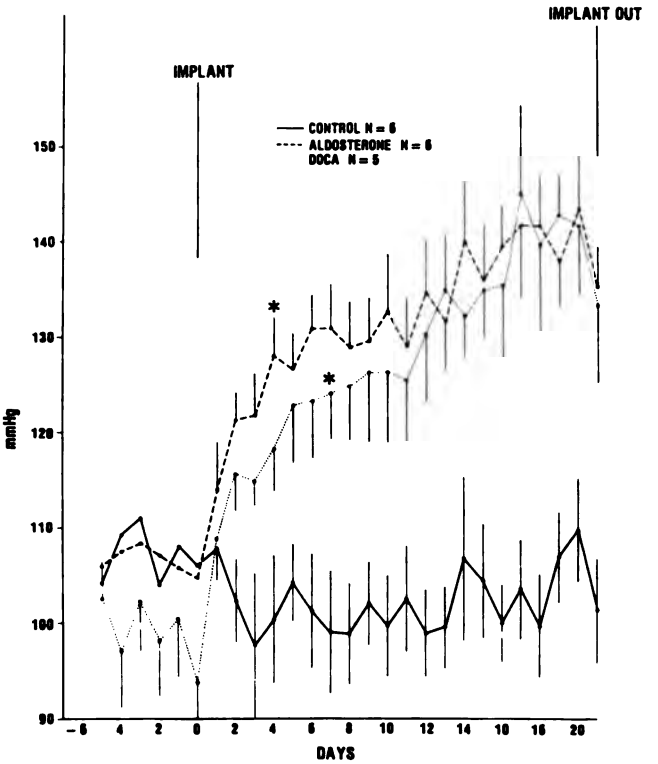

FIG. 1. Average mean arterial pressure of control, DOCA-hypertensive, and *d*-aldosterone-hypertensive pigs from 5 days before until 20 days after implantation of silastic (control), DOCA silastic, or *d*-aldosterone silastic. Pressures were measured using indwelling catheters advanced into the thoracic aorta via the left or right carotid artery. Data are presented as mean ± SEM. Mean pressures increased regularly in the DOCA- and *d*-aldosterone-implanted animals, and were significantly different from control by 4 days postimplantation with *d*-aldosterone (*) and 7 days postimplantation with DOCA (*).

conflicting. It is possible that the use of the racemic mixture, *d,l*-aldosterone, rather than the *d*-isomer alone may have been the source of some of the problems encountered in attempting to produce Aldo hypertension in an animal model (12). In several cases the isomer used is not stated. In addition, the number of daily injections, vehicle used, and doses administered differ considerably from one study to another. The present studies demonstrate than an intact adult animal will respond to the administration of Aldo as well as DOCA with a rapid and consistent increase in blood pressure. The Yucatan miniature boar therefore provides an excellent opportunity for comparative studies of the actions of DOCA and Aldo in an intact animal in its adult stage.

It has been reported that captopril, an inhibitor of kininase II (angiotensin-I-converting enzyme) had no effect on the development of DOCA–salt hypertension or on established DOCA–salt hypertension when administered in the drinking water of rats (13, 14). However, the same investigators subsequently reported a decrease in blood pressure in established aldosterone–salt hypertension and an attenuation of the hypertension when captopril was given during the developmental phase (15). These findings imply mechanistic differences in the hypertensive process resulting from the administration of these two steroids. The authors speculated a difference in the importance of the vasodilator kinins. Vascular reactivity studies in the rat

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PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 171, 83–87 (1982)

Concomitant Enhancement of B-Cell Mitogenesis and Inhibition of Antibody Synthesis by a Phorbol Ester (41481)

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Abstract. 12-*O*-Tetradecaroyl-phorbol-13-acetate (TPA), a potent tumor-promoting agent, was found to enhance proliferation of murine splenic lymphocytes in response to B-cell mitogens, while possessing no mitogenic activity by itself. At the same time, TPA inhibited the B mitogen-induced polyclonal responses as well as antigen-specific antibody responses of cultured murine spleen cells. Our results support the hypothesis that tumor promoters inhibit lymphocyte differentiation.

12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) is a potent tumor-promoting agent which enhances the oncogenic effect of carcinogens on mouse skin. To learn the basis of its tumor-promoting activity, the morphological and biochemical responses of cell cultures treated with TPA have been the subject of intense investigations. Early reports by Sivak and Van Duuren (1, 2) reported that the initial binding site of TPA is the cell membrane. Driedger and Blumberg (3) have shown that specific membrane binding activity for a series of phorbol esters correlates with their *in vivo* promoting activity. Subsequent to interaction with a membrane receptor TPA induces a series of biochemical events indicative of rapid cell proliferation that include increases in phospholipid synthesis and metabolism (4, 5), RNA and DNA synthesis (6, 7), polyamine synthesis through stimulation of ornithine decarboxylase activity (8), and uptake and transport of amino acids (9). TPA is mitogenic for a variety of cell types, including 3T3 fibroblasts (10), chick embryo myoblasts (11), and chondroblasts (12), hamster embryo fibroblasts (13), and peripheral blood lymphocytes obtained from primates (14). Proliferative effects of the T-lymphocyte mitogens concanavalin A and phytohemagglutinin on lymphocytes from bovine (15) and guinea pig (16) lymph nodes, and mouse spleens (17) were shown to be enhanced by TPA.

TPAs mitogenic properties have been linked to its ability to inhibit cell differentiation by possibly committing cells to continuous replication (18). Cohen *et al.* (19) showed that TPA prevents terminal differentiation of chick embryo myoblasts *in vitro*, and Yamasaki *et al.* (20) and Rovera *et al.* (21) showed similar effects on Friend erythroleukemia cells. It has also been shown that promoters inhibit morphological differentiation of cultured neuroblastoma cells (22). Contrary to the above reports several workers found that TPA may induce cell differentiation in erythroid cells of human promyelocytic leukemia cells (23–25).

In the present study we investigated the effects of TPA on *in vitro* lymphocyte responses utilizing assays which measure proliferation (mitogenesis) and terminal differentiation (antibody formation). We report that TPA is comitogenic when combined with the B-cell mitogens bacterial lipopolysaccharide (LPS) and muramyl dipeptide (MDP), while it inhibits the polyclonal antibody response induced by the same mitogens, as well as the antigen-specific response to sheep red blood cells (SRBC). These data support the hypothesis that TPA prevents terminal differentiation in uncommitted lymphocytes.

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Materials and Methods. *Animals.* BDF_1 (C57 Bl/6 × DBA/2) female mice 6–9 weeks of age were purchased from Jackson Laboratories, Bar Harbor, Maine.

Mitogens, antigens, and reagents. The phorbol ester, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), was obtained from D and L Biochemicals, Milwaukee, Wisconsin, and dissolved in acetone at a concentration of 100 μg/ml. Before use, TPA was diluted in culture media to the appropriate concentration. B-Cell mitogens used were bacterial lipopolysaccharide (*E. coli* 0127:08, Difco Labs., Detroit, Mich.) and muramyl dipeptide (*N*-acetylmuramyl-L-alanyl-D-isoglutamine, MDP, a gift of Dr. Gordon Jones, Syntex Corp., Palo Alto, Calif.) Sheep red blood cells (Gibco, Grand Island, N.Y.) were washed three times with media and suspended to a concentration of 1% for culture immunization and 20% for plaque-forming cell assays. Results for mitogenic assays are expressed as counts per minute [^{3}H]thymidine incorporation.

Culture media. Cell cultures were performed in RPMI 1640 containing 50 μg/ml gentamycin, 2×10^{-5} *M* 2-mercaptoethanol, bicarbonate buffer, L-glutamine, and 10% fetal calf serum.

Mitogenic assays. In vitro mitogenic assays were performed on splenic lymphocytes from BDF_1 mice. Cells (5×10^5) were incubated in 0.2 ml culture media in 96-well microculture plates (Costar, Cambridge, Mass.) for 3 days with mitogen and/or TPA at 37° in a humidified atmosphere containing 5% CO_2. For the final 18 hr of culture 1 μCi [^{3}H]thymidine was added per well. Cells were harvested on glass fiber filters using a Skatron Cell Harvester (Flow Labs., Rockville, Md.) and [^{3}H]thymidine uptake determined by liquid scintillation counting.

Plaque-forming cell cultures. In vitro cultures for antibody-forming cells were performed by the procedure of Mischell and Dutton (30). Splenic lymphocytes (5×10^6/0.5 ml) were incubated with SRBC (0.05 ml of a 1% solution) or polyclonal activator (LPS or MDP) for 4 days in 24-well culture dishes (Costar) on a rocking platform under a gas mixture of 83% N_2, 10% CO_2, and 7%

TABLE I. COMITOGENESIS OF TPA AND B-CELL MITOGENS MDP AND LPS

Mitogen (μg/ml)	TPA added (μg/ml) 0	10^0	0^-	10^{-2}	0^{-3}
MDP[a]					
100	9,791 ± 2249[b]	46,996 ± 6721	64,620 ± 8,211	31,331 ± 4,211	10,421 ± 667
10	8,450 ± 701	29,575 ± 3114	49,010 ± 2,661	21,125 ± 3,666	9,104 ± 722
1	4,361 ± 421	10,903 ± 1011	18,316 ± 3,112	8,286 ± 971	5,022 ± 311
0	1,775 ± 503	1,542 ± 311	1,700 ± 321	1,622 ± 493	1,200 ± 462
LPS[a]					
100	47,322 ± 5109	113,572 ± 9106	99,376 ± 10,100	85,179 ± 11,123	46,222 ± 6133
10	33,571 ± 2601	130,927 ± 15,461	137,641 ± 14,392	83,928 ± 11,262	40,285 ± 7111
1	25,806 ± 2140	118,708 ± 16,321	82,579 ± 9,132	64,515 ± 6,333	46,450 ± 3976
0	2,873 ± 191	2,433 ± 721	2,922 ± 681	2,544 ± 333	2,247 ± 201

[a] Three-day mitogenic assays were set up as in Materials and Methods. 100, 10, or 1 μg/ml MDP or LPS was added per well. Values are determined from quadruplicate cultures.
[b] Counts per minute [^{3}H]thymidine incorporation ± standard error of the mean.

O_2. Cells were fed daily with a nutritional cocktail (30).

Direct plaque-forming cells. Direct PFCs were determined by the slide modification of the Jerne plaque assay (30). Cultured cells (0.05 ml) were mixed with 0.5 ml of 0.6% agarose and 0.02 ml 20% SRBC and poured onto agarose-coated slides. Slides were incubated for 3 hr with 1/30 diluted guinea pig complement and the PFCs enumerated.

Results. Table I illustrates that TPA enhances cell division (as determined by uptake and incorporation of [^{3}H]thymidine) initiated by LPS or MDP at TPA doses of 10^{-3} to 1 μg/ml. The synergistic effect between LPS or MDP and TPA was evident if the reagents were added to the spleen cell cultures no longer than 6 hr apart, since we have found that if the interval was extended to 12 hr, the synergistic effects were not seen (data not shown). TPA was not mitogenic by itself at any dose tested, nor was any toxicity noted at the highest level used (1 μg/ml). MDP alone as mitogen routinely produced a stimulation of four to five times background while LPS produced a stimulation of 15–20 times. Because of TPAs marked effect on B-Cell proliferation, we wondered if the enhanced mitogenesis was reflected in an increased level of antibody synthesis. To measure TPAs effect on the antigen-specific and polyclonal antibody induction, it was added to *in vitro* cultures of splenic lymphocytes and the polyclonal (LPS or MDP induced) or antigen-specific (SRBC immunized) antibody synthesis was determined 4 days later using SRBCs as indicator cells. Results in Table II demonstrate that TPA is a potent inhibitor of both polyclonal and antigen-specific antibody responses. To determine whether early or late events in lymphocyte response to antigen were affected, TPA was added to cultures at 24-hr intervals, each culture receiving addition of TPA on either Day 0, 1, 2, 3, or 4 of culture. Results (Table III) indicate that addition of TPA to SRBC-immunized cultures from Days 0–3 significantly reduced the plaque-forming cell response to SRBC. TPA had no effect when added on Day 4, indicating that antibody secretion was not affected. Similar results were seen for the polyclonally induced plaque-forming cell response with the suppression significant on Days 0 and 1.

Discussion. The dual effects of B lymphocytes mitogens as activators of DNA synthesis and polyclonal antibody synthesis provided us with an opportunity to investigate the relationship between the requirement for DNA synthesis and subsequent induction of polyclonal immunoglobulin production. In an earlier study we demonstrated that LPS is capable of activating re-

TABLE II. SUPPRESSION OF ANTIGEN-SPECIFIC AND POLYCLONAL ANTIBODY SYNTHESIS BY TPA

	PFC/10^6 viable cells[b]	Percentage inhibition
Antigen specific		
SRBC[a]	406 ± 26	—
SRBC + TPA[e]	15 ± 3	96.3[c]
Polyclonal activation		
LPS[d]	191 ± 41	—
LPS + TPA	110 ± 31	42.2[c]
MDP[d]	116 ± 37	—
MDP + TPA	10 ± 3	91.4[c]
Controls		
TPA alone	11 ± 2	—
Media control	16 ± 6	—

[a] Cultures were immunized with 0.05 ml of a 1% solution of SRBCs.
[b] Direct PFCs ± SEM were determined using SRBCs as indicator cells.
[c] Significantly different from control as determined by Student's t test, $P < 0.01$.
[d] LPS (10 μg/ml) or MDP (100 μg/ml) were added to cultures at initiation.
[e] TPA was added to a final concentration of 1 μg/ml.

TABLE III. EFFECT OF ADDITION OF TPA AT VARIOUS TIMES DURING CULTURE

1 μg/ml TPA added on Day:[a]	PFC/10[6] viable cells[b]		
	SRBC specific[c]	polyclonal activator[d]	
		MDP	LPS
0	19 ± 2	11 ± 1	167 ± 19
1	28 ± 1	21 ± 5	196 ± 36
2	50 ± 10	135 ± 40	265 ± 25
3	169 ± 10	153 ± 28	333 ± 10
4	377 ± 65	108 ± 44	308 ± 39
None	341 ± 16	105 ± 44	465 ± 20

[a] Suppression significant when TPA added on Days 0, 1, 2, 3 to SRBC and Days 0, 1 in polyclonal activation, $P < 0.01$.

[b] Direct PFC/10[6] viable cells ± SEM determined on Day 4 using SRBCs as indicator cells.

[c] Cultures were immunized with SRBCs on Day 0.

[d] MDP (100 μg/ml) or LPS (10 μg/ml) was added on Day 0.

sponsive B cells to polyclonal antibody production without significant cellular proliferation (26). Both hydroxyurea and cytosine arabinoside, known inhibitors of DNA synthesis, failed to inhibit the LPS-induced polyclonal response, substantiating a dissociation between proliferative and polyclonal responses to LPS. In the present study addition of TPA to splenic cultures enhanced LPS and MDP mitogenicity but caused profound suppression of antibody synthesis. Our results support the hypothesis that TPA inhibits early stages of lymphocyte differentiation into antibody-forming cells, as the most profound suppression of antibody synthesis was seen when TPA was added on Days 0 and 1 of the culture. It appears unlikely that TPA is affecting the synthesis of products of the differentiated state (i.e., antibody), since TPA does not block antibody synthesis or secretion in murine myeloma cells which are already terminally differentiated (11) or affect antibody-forming cell cultures in these experiments when added on Day 4.

It is conceivable that TPAs effects on lymphocyte differentiation contribute to its tumor-promoting activity. In support of this thesis, Keller (27) has shown that TPA inhibits the killing of tumor cells by macrophages, and Mastro and Mueller (28) and Fish *et al.* (29) demonstrated inhibition of the mixed lymphocyte response by TPA.

An alternative possibility would encompass the current knowledge of the effects of TPA on accessory cells (macrophages). Mizel *et al.* (31) reported that TPA stimulates lymphocyte-activating factor (LAF or Interleukin 1) production in the macrophage cell line P388D$_1$, and Earrar *et al.* (32) showed enhanced production of Interleukin 2 (IL2, formerly T-cell growth factor) by T cells in the presence of phorbol ester. IL2 is known to result from IL1 production from macrophages (33). It is conceivable that TPA stimulates these and other factors which enhance the mitogenesis of B lymphocytes. The inhibition of antibody synthesis observed may be a secondary effect resulting from the activation suppressor cells by these macrophage and/or T-cell factors. Further work on the subcellular events involved will answer these questions.

1. Sivak A, Mossman BT, Van Duuren BL. Activation of cell membrane enzymes in the stimulation of all division. Biochem Biophys Res Commun 46:605, 1972.
2. Sivak A, Raym F, Van Duuren BL. Phorbol ester tumor promoting agents and membrane stability. Cancer Res 29:624, 1969.
3. Driedger PE, Blumberg P. Specific binding of phorbol ester tumor promoters. Proc Natl Acad Sci USA 77:567, 1980.
4. Levine L, Hassid A. Effects of phorbol-12,13-diesters on prostaglandin activity in canine kidney (MDCK) cells. Biochem Biophys Res Commun 79:477, 1977.
5. Suss R, Kreibich G, Kinzel V. Phorbol esters as a tool in cell research. Eur J Cancer 8:299, 1972.
6. Peterson AR, Mondal S, Brankow DW, Thon W, Heidelberger C. Effects of promoters on DNA synthesis in C^{3}H 10T1/2 mouse fibroblasts. Cancer Res 37:3223, 1977.
7. Suss R, Schuster A. Tumor promoting Crotonoil factor tetradecanoyl-phorbolacetate stimulates thymidine incorporation in normal and Con-A stimulated thymocytes. Experentia 30:81, 1974.
8. O'Brien TG, Simisman RC, and Boutwell RK. Induction of the polyamine-biosynthetic enzymes in mouse epidermis by tumor promoting agents. Cancer Res 35:1662, 1975.
9. Kensler TW, Wertz PW, and Mueller GC. Inhibi-

tion of phorbol ester-accelerated amino acid transport in bovine lymphocytes. Biochim Phys Acta 585:43, 1979.
10. Diamond L, O'Brien TG, Rovera G. Inhibition of adipose conversion of 3T3 fibroblasts by tumor promoters. Nature (London) 269:247, 1977.
11. Diamond L, O'Brien TG, Rovera G. Tumor promoters: Effects on proliferation and differentiation of cells in culture. Life Sci 23, 1978.
12. Pacifici M, Holtzer H. Effects of a tumor promoting agent on chondrogenesis. Amer J Anat 150:207, 1977.
13. Lankas GR Jr, Baxter CS, Christian R. Effect of tumor promoting agents on mutation frequencies in cultural V79 chinese hamster cells. Mutat Res 45:153, 1977.
14. Abb J, Bayliss G, Deinhardt F. Lymphocyte activation by the tumor promoting agent 12-O-tetradecanoyl-pherbol-13-astate (TPA). J Immunol 122:1639, 1979.
15. Mastro A, Mueller GC. Synergistic action of phorbol esters in mitogen-activated lymphocytes. Exp Cell Res 88:40, 1974.
16. Rosenstreich DL, Mizel S. Signal requirements for T-lymphocyte activation. I. Replacement of macrophage function with phorbol myristate acetate. J Immunol 123:1749, 1979.
17. Fish LA, Baxter CS, Bash JA. Comitogenicity of tumor promoting. J Toxicol Appl Pharmacol 48:A151, 1979.
18. Boutwell RK. The function and metabolism of promoters of carcinogenesis. CRC Crit Rev Toxicol 2:419, 1974.
19. Cohen R, Pacifici M, Rubinstein N, Biehl J, Holtzer H. Effect of tumor promoters on myogenesis. Nature (London) 266:538, 1977.
20. Yamasaki H, Fibach E, Nudel U, Weinstein IB, Rifkind RA, Marks PA. Tumor promoters inhibit spontaneous and induced differentiation of murine erythroleukemia cells in culture. Proc Natl Acad Sci USA 74:3451, 1977.
21. Rovera G, O'Brien TG, Diamond L. Tumor promoters inhibit spontaneous differentiation of Friend erythroleukemia cells in culture. Proc Natl Acad Sci USA 74:2894, 1977.
22. Ishii D, Fibach E, Yamasaki H, Weinstein IB. Tumor promoters inhibit morphological differentiation in cultured mouse neuroblastoma cells. Science 200:556, 1978.
23. Rovera G, O'Brien TG, Diamond L. Induction of differentiation in human promyelocytic leukemic cells by tumor promoters. Science 204:868, 1979.
24. Miao R, Fieldsteel A, Fodge D. Opposing effects of tumor promoters on erythroid differentiation. Nature (London) 274:271, 1978.
25. Huberman E, Callaham M. Induction of terminal differentiation in human promyelocytic leukemia cells by tumor promoting agents. Proc Natl Acad Sci USA 76:1293, 1979.
26. Poe W, Michael J. Separation of the mitogenic and antigenic responses to bacterial lipopolysaccharide. Immunology 30:241, 1976.
27. Keller R. Suppression of anti-tumor defense mechanisms by phorbol esters. Nature (London) 282:729, 1979.
28. Mastro A, Mueller GC. Inhibition of the mixed lymphocyte proliferative response by phorbol esters. Biochim Biophys Acta 517:246, 1978.
29. Fish LA, Baxter CS, Bash JA. Parallel order of reactivity on murine cells mezereine and phorbol esters toward the mixed lymphocyte response and promotion of tumor regenesis. Toxicol Appl Pharmacol 59:173–176, 1981.
30. Mischell R, Dutton R. Immunization of dissociated spleen cell cultures from normal mice. J Exp Med 126:423, 1967.
31. Mizel SB, Rosenstreich DL, Oppenheim JJ. Pherbol myristate acetate stimulates LAF production by the macrophage cell line P388D. Cell Immunol 40:320, 1978.
32. Farrar JJ, Mizel SB, Fuller-Farrar J, Farrar WL, Hilfilter MI. Macrophage-independent activation of helper T-cells. I. Production of Interleukin 2. J Immunol 125:793, 1980.
33. Oppenheim JJ, Mizel SB, Melter MS. Biological effects of lymphocyte and macrophage-derived mitogenic amplification factors. *In* Cohen S, Pick E, Oppenheim JJ, eds. Biology of the Lymphokines. New York, Academic Press, p291, 1979.

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Cobalamin (Vitamin B_{12}) Analogs Are Absent in Plasma of Fruit Bats Exposed to Nitrous Oxide[1] (41482)

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Abstract. Fruit bats are an animal model for the neurologic damage which occurs in vitamin B_{12}-deficient humans. Cobalamin (vitamin B_{12}) analogs were not detected in the plasma of fruit bats treated with nitrous oxide (N_2O), which inactivates cobalamin. This observation does not lend support to the suggestion that the neurological changes associated with cobalamin inactivation by N_2O and/or cobalamin deficiency per se may be related to the accumulation of cobalamin analogs. However, although the plasma of control fruit bats lacked analogs, we did find analogs in their livers, at levels about 10% of total liver corrinoids, similar to human liver analog levels.

It was recently suggested that physiologically inactive cobalamin (vitamin B_{12}) analogs are present in mammalian plasma (1) and tissues (2). If present, these analogs may have considerable importance, for they could exacerbate the effects of true cobalamin deficiency (3), such as the neurological changes. Kondo *et al.* (4) reported that exposure of rats to nitrous oxide (N_2O), which inactivates cobalamin (5), also results in the conversion of cobalamin to analogs in the liver, but that the animals do not develop neurological changes. When fruit bats are fed a cobalamin-free diet, they develop neurological changes similar to those seen in human cobalamin deficiency, within 9 to 12 months (6), but this is not associated with the presence of cobalamin analogs (7). As gross neurological changes occur rapidly in fruit bats exposed to N_2O (our unpublished data), the purpose of the present study was to determine whether cobalamin analogs played a role in the development of the N_2O-induced neurological changes in these animals.

Materials and Methods. *Experimental animals.* Fruit bats (*Rousettus aegyptiacus*) were captured in the wild and rendered cobalamin-deficient on an all-fruit diet (6). To prevent other vitamin deficiencies from developing, 0.2 ml of a cobalamin-free oral vitamin preparation (Abidec, Parke-Davis) was administered every 2 weeks in a dose containing 100 IU vitamin D, 0.25 mg thiamine, 0.01 mg riboflavin, 0.12 mg niacin, and 12.5 mg ascorbic acid. Bats maintained on this diet became cobalamin-deficient after 9 to 12 months. Control bats received intramuscular injection of 0.5 μg cyanocobalamin per 100 g body weight every 2 weeks.

Exposure to nitrous oxide. Bats were exposed to an atmosphere of 50% O_2/50% N_2O for 90 min every day for 3 weeks in a specially constructed cabinet in which CO_2 and water vapor were controlled. For 22.5 hr a day, the bats breathed room air in their usual aviary, in which they had ample room to fly.

Measurement of cobalamin analogs. Blood was drawn by cardiac puncture into heparinized tubes. The plasma was separated by centrifugation and stored at −20°. Plasma cobalamin analogs were determined by the Lau *et al.* coated charcoal radioisotope dilution technique (8) as modified by Kolhouse *et al.* (1), using salivary R-binder as ligand to measure total corrinoids, and pure intrinsic factor (IF) as

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ligand to measure intact cobalamins, based on the facts that IF has a very low affinity for cobalamin analogs other than intact cobalamins, whereas the affinity of R-binders for these analogs is so much greater that it binds the totality of intact cobalamins plus other corrinoids (1, 9).

The R-binder used was human saliva, and the IF used was a gift from Becton-Dickinson Immunodiagnostics (Orangeburg, N.Y.) of pure hog IF prepared by affinity chromatography. The process for extraction from serum of cobalamin and analogs was that used by Kolhouse *et al.* (1), as was the amount of cyanide and BSA (bovine serum albumin) added. Separation of bound from free cobalamin was with albumin-coated charcoal prepared as described by Lau *et al.* (8). The reference standard was USP (United States Pharmacopeia) Cyanocobalamin Standard, purchased from the USP (Washington, D.C.). We have used this identical methodology to study cobalamin and analog levels in human serum, red cells, brain, liver, and bile (10–12). The amount of analogs present is thus represented by subtracting from the total corrinoids detected when R-binder is used as ligand at pH 9, the quantity of intact cobalamins measured at pH 9 with IF as ligand (1).

Results. The results are shown in Table I.

The group of four cobalamin-replete fruit bats received intramuscular injections of cyanocobalamin every 2 weeks during the 2 months they were in captivity. Serum cobalamin levels were normal, varying from 1536 to 2781 pg/ml when assayed with IF as ligand. No significant amounts of cobalamin analog were present in the plasma of these bats, the analog ranging from 0 to 126 pg/ml.

The bats rendered cobalamin deficient had received the cobalamin-free diet for 12 to 21 months and all were severely cobalamin deficient (plasma cobalamin = 1–91 pg/ml). No significant amounts of cobalamin analog could be detected in deficient animals exposed to N_2O (range = 0–18 pg/ml), or those not so exposed (range = 0–33 pg/ml).

Discussion. In all fruit bats studied, cobalamin analogs were absent from the plasma or present in negligible amounts only. In the bats rendered cobalamin deficient by dietary means only, the results were similar to those reported by Green and Jacobsen (7). Cobalamin analogs were also not detected in the plasma of bats exposed to N_2O, which suggests that analogs do not play a role in the development of the severe neurological changes that accompany N_2O exposure in the bat. Furthermore, cobalamin analogs were detected in small amounts

TABLE I. PLASMA LEVELS OF COBALAMINS AND ANALOGS (pg/ml) IN FRUIT BATS[a]

Group.	No.	Cobalamins	Total corrinoids	Analogs
Cobalamin-replete	1	2610	2713	103
	2	2781	2786	5
	3	1536	1490	0
	4	2747	2873	126
Cobalamin-deficient	5	19	0	0
	6	26	59	33
	7	16	0	0
	8	58	77	19
Cobalamin-deficient exposed to N_2O	9	1	0	0
	10	23	41	18
	11	91	60	0
	12	26	1	0

[a] Assayed by radioisotope dilution assays using as ligand pure intrinsic factor (IF) for cobalamins and salivary R-binder for total corrinoids (cobalamins + analogs).

in only two of four fruit bats cobalamin replete from intramuscular cobalamin injections every 2 weeks; this slightly differs from the finding by Green *et al.* (13) of quantity of analogs present in serum of fruit bats injected with 100 ng cyanocobalamin weekly (but not in other fruit bats).

It is not clear why cobalamin analogs appear in the tissues of N_2O-treated rats (4) but not in the plasma of bats. This may represent a species difference, for there is evidence that the bat and the rat respond differently to N_2O exposure: N_2O causes severe neurological changes in the bat but none in the rat, and the deoxyuridine (dU) suppression test (14) is abnormal in the N_2O-exposed rat (15) but not in the bat (our unpublished observations). Another possibility is that the length of exposure to N_2O may be critical, for Kondo and co-workers (4) exposed rats continuously to N_2O for periods ranging from 30 min to 38 hr, while the bats in the present study were exposed for 90 min daily for 3 weeks. It is also possible that analogs present in tissues may be cleared more rapidly from the plasma of the bat than of the rat.

The term "analog" as used in the present study does not refer to any specifically isolated form or forms of corrinoids, but is used to describe molecules which have a corrin nucleus and therefore attach to R-binders as does vitamin B_{12}, but in addition have a lesser affinity than cyanocobalamin for IF and/or a much greater affinity than cyanocobalamin for R-binder.

When we studied the livers of 4 control and 6 nitrous-oxide treated bats, we found the control bats had a mean of 65.8 ± 16.1 ng total corrinoid/g liver, a mean of 59.9 ± 15.7 ng cobalamin/g liver, and a mean of 5.9 ± 2 ng analog/g liver. Thus, although normal fruit bats lack cobalamin analogs in their serum, such analogs are present in their livers, suggesting more rapid clearance of analogs from serum into liver in bats than in humans (10–12). Livers from the 6 N_2O-treated bats had a mean of 1.3 ± 1.3 ng total corrinoid/g liver, a mean of 1.5 ± 0.5 ng cobalamin/g liver, and no measurable analog in their livers.

1. Kolhouse JF, Kondo H, Allen NC, Podell ER, Allen RH. Cobalamin analogues are present in human plasma and can mask cobalamin deficiency because current radioisotopic dilution assays are not specific for true cobalamin. New Engl J Med 299:785–792, 1978.
2. Kondo H, Kolhouse JF, Allen RD. Presence of cobalamin analogues in animal tissues. Proc Nat Acad Sci USA 77:817–821, 1980.
3. Kolhouse JF, Allen RH. Absorption, plasma transport, and cellular retention of cobalamin analogues in the rabbit. J Clin Invest 60:1381–1392, 1977.
4. Kondo H, Osborne ML, Kolhouse JF, Binder MJ, Podell ER, Utley CS, Abrams RS, Allen RH. Nitrous oxide has multiple deleterious effects on cobalamin metabolism and causes decreases in activities of both mammalian cobalamin-dependent enzymes in rats. J Clin Invest 67:1270–1283, 1981.
5. Blackburn R, Kyaw M, Swallow AJ. Reaction of Cob(I)alamin with nitrous oxide and Cob(III)alamin. J Chem Soc, Faraday Trans 73:250–255, 1977.
6. Green R, van Tonder SV, Oettle GJ, Cole G, Metz J. Neurological changes in fruit bats deficient in vitamin B_{12}. Nature (London) 254:148–150, 1975.
7. Green R, Jacobsen DW. No discrepancy between vitamin B_{12} radioassays using purified intrinsic factor or R binder in bats developing cobalamin deficient neuropathy. Clin Res 28:74A, 1980.
8. Lau KS, Gottlieb C, Wasserman LR, Herbert V. Measurement of serum vitamin B_{12} level using radioisotope dilution and coated charcoal. Blood 26:202–214, 1965.
9. Gottlieb CW, Retief FP, Herbert V. Blockade of vitamin B_{12}-binding sites in gastric juice, serum and saliva by analogues and derivatives of vitamin B_{12} and by antibody to intrinsic factor. Biochim Biophys Acta 141:560–572, 1967.
10. Kanazawa S, Herbert V. Vitamin B_{12} analog content in human red cells, liver, and brain. Clin Res 30:504A, 1982.
11. Herbert V, Kanazawa S, Drivas G, Foscaldi R, Manusselis C, Herzlich B, Colman N, van der Westhuyzen J, Fernandes-Costa F, Metz J. Evidence for presence of stable vitamin B_{12} analogs in serum of bushmen on "natural" diets, and in liver of Americans, and analog absence in red cells of Americans, and fruit bat serum (even after vitamin B_{12} supplementation). In: Proceedings, 19th Congress of the International Society for Hematology, Budapest, August 1–7, p 236, 1982.
12. Kanazawa S, Herzlich B, Drivas G, Manusselis C, Herbert V. Removal of cobalamin analog in bile may be the primary purpose of the enterohepatic

circulation of vitamin B_{12}. Clin Res 30:687A (1982).

13. Green R, Jacobsen DW, Sommer C. Cobalamin depleted fruit bats show discrepancies between radioassays using purified intrinsic factor or R-binder after they are given a cyanocobalamin supplement. Blood 58 (Suppl. 1):27a, 1981.
14. Metz J, Kelly A, Swett VC, Waxman S, Herbert V. Deranged DNA synthesis by bone marrow from vitamin B_{12}-deficient humans. Brit J Haematol 14:575–592, 1968.
15. Deacon R, Lumb M, Perry J, Chanarin I, Minty B, Halsey MJ, Nunn JF. Selective inactivation of vitamin B_{12} in rats by nitrous oxide. Lancet 2:1023–1024, 1978.

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Therapeutic Concentrations of Antineoplastic Agents Diminish Interferon Yields (41483)

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Abstract. Interferon production was studied in the presence of therapeutic concentrations of five antineoplastic agents. None of the drugs studied grossly altered the production of interferon by fibroblasts. Virus-induced mononuclear cells, in contrast, produced significantly less interferon after prior exposure to vincristine and produced no detectable interferon after prior exposure to 6-MP. These studies establish that interferon yields *in vitro* may be diminished by therapeutic concentrations of antineoplastic agents, and necessitate *in vivo* studies to determine if this alteration in interferon production can help explain the increased severity of certain viral infections in patients with neoplastic diseases.

Patients with certain forms of malignant disease are known to be at increased risk for serious viral infections (1). Because of this fact, we have questioned whether interferon production may be diminished in malignant disease particularly by the chemotherapeutic agents used to treat neoplastic processes.

As a preliminary to investigations *in vivo*, we have studied the effects of various antineoplastic agents on the ability of human cells to produce interferon *in vitro*.

This report reviews our experience with the production of interferon in the presence of five antineoplastic agents. We have found that therapeutic concentrations of these agents can influence interferon production in human cells, especially mononuclear cells. We suggest *in vivo* studies be done to determine if this phenomenon occurs in cancer patients receiving chemotherapy.

Materials and Methods. *Cells. Fibroblasts.* Human foreskin fibroblasts (FF cells) originally prepared in our own laboratory were used for these experiments. Fibroblasts were grown to confluence in 25-cm^2 plastic screw-cap flasks prior to interferon induction.

Mononuclear leukocytes. Human leukocytes were obtained from the American Red Cross as the platelet-rich fraction of human blood. These preparations were tested within 24 hr of procurement. Upon receipt in the laboratory, the cell suspension was subjected to centrifugation on Ficoll–Hypaque for 45 min at 1000 rpm. The mononuclear cell layer was then harvested, washed three times with phosphate-buffered saline (PBS), and adjusted to a concentration of 1×10^7 cells/ml in Newman Tytell medium containing 10% bovine fetal serum (BFS).

Antineoplastic agents. Antineoplastic agents were obtained from the following sources: 5-fluorouracil from Roche Laboratories, Nutley, New Jersey; adriamycin from Farmitalia, SPS, Italy; vincristine from Eli Lilly, Indianapolis, Indiana; 6-mercaptopurine (6MP) from Burroughs Wellcome, Research Triangle Park, North Carolina; and methylprednisolone from Upjohn Company, Kalamazoo, Michigan.

All solutions were freshly prepared immediately prior to use. The concentrations employed in these experiments were chosen to approximate those present in the serum immediately following administration to humans (2–5).

Inducing agents. Complexed polyinosinic–polycydidylic acids (I:C) were obtained either in liquid or powdered form and were diluted just prior to use in phosphate-

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buffered saline (PBS) containing calcium and magnesium.

Diethylaminoethyl dextran (DEAE-D) was dissolved in water, in a concentrated form, and frozen at −20°. Immediately prior to use, an aliquot of DEAE-D was added to the I:C solution so that the final concentration of the inducing solution was 10 μg/ml of I:C and 100 μg/ml of DEAE-D.

Newcastle disease virus (NDV) was grown in eggs using a laboratory strain that had been serially passed through many generations.

Induction of interferon. Preliminary experiments demonstrated that the various concentrations of drugs employed were not toxic to the fibroblast monolayers (as evidenced by lack of morphologic changes) and would not induce interferon.

FF cells were grown to confluence in screw-cap flasks. At the initiation of the experiment three flasks of fibroblasts were exposed to L-15 media containing 2% bovine fetal serum, 1 mg/ml dextrose, 30 μg/ml glutamine, 90 μg/ml arginine, 150 u/ml penicillin, 250 μg/ml of streptomycin, and an appropriate concentration of the antineoplastic agent to be tested. Control flasks and simultaneous exposure flasks were treated with this same media without the antineoplastic agent during this 24-hr period. After the preliminary exposure, I:C induction was accomplished by adding 2 ml of the inducing solution containing the individual antineoplastic agents in the concentrations noted to both pretreatment and simultaneous exposure flasks. Control flasks were provided with 2 ml of the inducing solution without the antineoplastic agents.

All flasks were exposed to the inducing solutions for 3 hr and then washed with PBS. Subsequently, 2 ml of L-15 media supplemented as described above was added. Individual antineoplastic agents in the appropriate concentrations were added to the media of the test flasks (both pretreated and simultaneously treated), but control flasks contained the same media without such agents. The flasks were incubated overnight and the media was then harvested. All solutions were dialyzed for 24 hr against 100 vol of PBS to remove the antineoplastic agent, harvested, and frozen at −80° until assayed. Control and test inductions were performed simultaneously under identical circumstances save for the presence of the drug in the test solutions. All inductions were performed in triplicate.

In the case of induction with virus, duplicate flasks were prior treated as described above. At the appropriate time, the prior-treated flasks and unexposed flasks were suctioned and exposed to NDV in a concentration of 100 EID_{50} per cell for 1 hr. At the conclusion of this exposure, the two prior-treated flasks as well as the simultaneous exposure flasks were covered with 2 ml of the supplemented L-15 containing the antineoplastic agents. Duplicate control flasks, which had been exposed to virus but not to the test drugs, were covered with a similar quantity of supplemented L-15 without any antineoplastic drug.

Incubation was then carried out for 24 hr after which the media was harvested and dialyzed against 100 vol of 0.1 *M* citric acid for 4 days. The dialysis bath was then changed to PBS for 24 hr. Subsequently, the solutions were harvested and ultracentrifuged at 25,000 rpm for 90 min. Supernatants were harvested and stored at −80° until assayed.

Induction of interferon in mononuclear leukocytes was accomplished as follows: cells were adjusted to a concentration of 10^7 cells/ml in Newman Tytell media supplemented with 10% BFS, 250 u/ml of penicillin, and 150 μg/ml of streptomycin. Tubes used for the pretreatment experiments were exposed to media containing the appropriate concentration of antineoplastic agent for 24 hr at 37° in a 5% CO_2 incubator. Other tubes were simultaneously incubated in identical media save for the absence of the drugs.

Freshly prepared media was then added to all cells: the pretreatment and simultaneous exposure tubes receiving media containing the appropriate drug and the control tubes receiving the media without the drugs. NDV in a concentration of 100 EID_{50}/cell was added to each tube and incubation carried out for 24 hr in an incubator containing 5% CO_2. At the conclu-

tion of the incubation period the cells were centrifuged, the supernatant harvested, and the media dialyzed against citric acid for 4 days and PBS for 1 day. The media was then harvested, centrifuged for 90 min at 25,000 rpm, and frozen at −80° for later assay.

Viability counts were performed by the trypan blue exclusion method prior to discarding all cells. For all experiments at least 90% of the cells were viable at the conclusion of the experiment and the difference in viability between test and control tubes was always less than 10%.

Interferon assays. Interferon was assayed using a microtiter method which employed vesicular stomatitis virus as challenge and was performed on foreskin fibroblasts. This assay has been described previously (6, 7) and includes a standard assayed against NIH reference standard G-023-901-527.

Results. Tables I and II describe our results. No differences were appreciated between the quantity of interferon produced by fibroblasts in the presence of the antineoplastic agents or in the absence of these same drugs when I:C was used as the inducer. When fibroblasts were induced using NDV, only pretreatment with adriamycin was associated with any consistent reduction in interferon yield, but this reduction was of a minimal degree and its significance seemed slight. Methylprednisolone, 5FU, 6MP, and vincristine had no adverse effect on virus-induced interferon in fibroblasts even with pretreatment. All results were repeatedly reproduced in separate testing.

In contrast to the results with fibroblasts,

TABLE I. THE EFFECTS OF ANTINEOPLASTIC AGENTS ON INTERFERON PRODUCTION IN FIBROBLASTS

		Interferon titers								
		Antineoplastic agent present						Antineoplastic agent absent		
		Prior exposure			Simultaneous exposure					
Agent	Concentration	1	2	3	1	2	3	1	2	3
		I:C induction								
Vincristine	(0.08 μg/ml)									
Test 1		200	200	400	400	400	400	200	200	200
Test 2		3200	3200	3200	1600	3200	3200	3200	3200	3200
6 Mercaptopurine	(3.2 μg/ml)									
Test 1		200	200	200	200	400	400	400	400	400
Test 2		800	800	1600	800	800	800	800	800	1600
Adriamycin	(0.40 μg/ml)									
Test 1		800	1600	1600	400	800	800	1600	1600	1600
Test 2		200	200	200	200	200	200	200	200	200
5 Fluorouracil	(40.0 μg/ml)									
Test 1		800	800	1600	800	800	800	800	800	800
Test 2		1600	1600	1600	400	800	1600	400	800	800
Methylprednisolone	(80.0 μg/ml)									
Test 1		200	400	400	200	400	400	400	400	400
Test 2		400	800	800	400	400	800	400	400	800
		NDV induction								
Vincristine	(0.08 μg/ml)	1600	3200	—	3200	3200	—	3200	3200	—
6 Mercaptopurine	(3.2 μg/ml)	3200	3200	—	1600	3200	—	1600	3200	—
Adriamycin	(0.40 μg/ml)									
Test 1		1600	1600	—	800	3200	—	3200	3200	—
Test 2		1600	1600	—	3200	6400	—	6400	6400	—
Test 3		3200	-	—	6400	—	—	6400	—	—
Test 4		1600		--	3200	—	—	3200	—	—
5 Fluorouracil	(40.0 μg/ml)	800	1600	—	800	1600	—	1600	1600	—
Methylprednisolone	(80.0 μg/ml)	6400	6400	—	6400	6400	—	3200	6400	—

TABLE II. THE EFFECTS OF ANTINEOPLASTIC AGENTS ON INTERFERON PRODUCTION IN HUMAN MONONUCLEAR CELLS[a]

		Interferon titers					
		Antineoplastic agent present				Antineoplastic agent absent	
		Prior exposure		Simultaneous exposure			
Agent	Concentration	1	2	1	2	1	2
Vincristine	(0.08 μg/ml)						
Test 1		100	100	400	400	800	800
Test 2		400	800	800	1600	1600	1600
Test 3		200	200	—	—	800	800
6-Mercaptopurine	(3.2 μg/ml)						
Test 1		<100	<100	400	800	1600	1600
Test 2		<100	<100	400	800	800	800
Test 3		<100	<100	—	—	1600	1600
Adriamycin	(0.40 μg/ml)						
Test 1		1600	1600	800	1600	1600	1600
Test 2		800	800	400	800	800	800
5-Fluorouracil	(40.0 μg/ml)						
Test 1		800	1600	1600	1600	1600	1600
Test 2		800	1600	400	800	800	800
Methylprednisolone	(80.0 μg/ml)						
Test 1		400	800	400	800	800	800
Test 2		800	1600	800	1600	1600	1600

[a] NDV induction.

vincristine consistently lowered interferon yields when the leukocytes were pretreated with this drug, and 6MP, under the same conditions, virtually eliminated detectable interferon production. Both of the differences were significant ($P < 0.01$ by Student's t test). Simultaneous exposure for both drugs resulted in a diminished yield in three of the four tests. Adriamycin, methylprednisolone, and 5-fluorouracil failed to diminish mononuclear cell interferon yields. Again the results were reproducible in separate testing.

[Titration of NDV performed by hemagglutination before and after induction in both fibroblasts and mononuclear cells demonstrated that no change in virus concentration occurred during induction in either test or control flasks.]

Discussion. This report demonstrated that antineoplastic agents can influence interferon production by human cells *in vitro*. The observed alterations in interferon production, however, related not only to the particular drug in question but also to the cell type and the inducer.

We found the production of fibroblast interferon after I:C induction was unaltered by any of the antineoplastic agents studied. Furthermore, while adriamycin appeared to diminish the yield of fibroblast interferon if the cells were pretreated with the drug for 24 hr prior to induction with NDV, the difference in interferon yield was minimal. None of the other drugs studied affected virus-induced interferon production by fibroblasts. Thus, it seems fibroblasts were relatively resistant to the effects of the antineoplastic agents, at least as regards interferon production.

In contrast to the observations made with fibroblasts, vincristine and 6-MP lowered the quantity of interferon produced by mononuclear cells after viral induction. The reduced yields, however, were consistently seen only when mononuclear cells were pretreated with the antineoplastic agent. Prior treatment with vincristine reduced the quantity of interferon produced by as much as 88% and pretreatment with 6-MP virtually eliminated detectable interferon production.

In the circumstances where diminished interferon yields were demonstrated the question arises as to whether these effects occurred because of alterations in the host cell or because of effects on the virus. Virus titrations were performed by hemagglutination during induction and no viral proliferation could be documented under these circumstances. While infectivity measurements would have been a more reliable means of assessing viral proliferation, the fact that viral reproduction may not occur in this system suggests alterations in the cell are responsible for the observed diminution in interferon yields. Furthermore, if the effects were on the virus it would have been expected that the drugs would have *decreased interferon yields in both types of cells* used rather than just one as we described. It therefore seems likely that the diminished yields were due to alterations in the cell induced. It is probable that cell macromolecular synthetic capacities were compromised in those circumstances where diminished yields were documented. This hypothesis will be verified in future studies. Furthermore, it is likely that pretreatment permits the compromise in synthetic functions to occur before interferon production begins while simultaneous treatment permits the initiation of interferon synthesis before the drugs can affect the cell. Previous studies have provided a precedent for the assumption that protein-synthesizing capacities may be impaired at least by 6MP. Thus, Tidd and Paterson (8) have described alterations in RNA due to 6MP, and as both RNA (9, 10) and protein synthesis (11) are required for interferon production, chemicals which alter RNA could adversely influence interferon yield.

The fact that 6MP and vincristine altered production of interferon in mononuclear cells, but not fibroblasts, likely relates to the specific effects these drugs have on individual cell types. Thus, 6MP affects primarily leukemic cells (12) and vincristine affects differing cell types including those derived from lymphoidal origin (12).

The concentrations of chemotherapeutic agents used in these experiments were purposely selected to approximate the highest or peak therapeutic concentrations achieved in man. These high concentrations were used to maximize our opportunity to demonstrate any effect on interferon synthesis by these drugs. We have shown that at these peak therapeutic concentrations 6MP and especially vincristine can influence interferon production in mononuclear cells. Since, however, drug concentrations fall as the drug is eliminated by metabolism or excretion, it is now necessary to perform *in vivo* studies to find if the effect on interferon synthesis reported here can be demonstrated in the body. Further studies are currently in progress in our laboratory to find the minimum concentrations necessary to diminish interferon yields from mononuclear cells and to determine the duration of drug exposure necessary to affect this lessened production of interferon.

These studies offer some support to the hypothesis that the increased severity of viral infections occasionally observed during the course of neoplastic diseases could be at least in part due to diminished capacity of the patient to produce interferon in the presence of antineoplastic drugs. *In vivo* studies as mentioned above, however, will be necessary to confirm this hypothesis.

While no other comprehensive study similar to this investigation has been carried out on human cells, other relevant investigations deserve comment. Kilbourne *et al.* (13) using eggs, Postic *et al.* (14) using rabbits, and Talas and Stoger (15) using mice, all demonstrated diminished interferon production in the presence of steroids. In contrast, DeSomer *et al.* (16) working in rats found steroids failed to depress interferon levels and Mendelson *et al.* (17) found steroids actually increased interferon levels in mice.

As regards studies in tissue culture models, DeMaeyer and DeMaeyer (18) working on rat tumor cells found steroids depressed interferon production, but Adolf and Swetly (19) have recently found that various steroids can augment interferon production of human lymphoblastoid cells when induced by viruses.

Havell and Vilcek (20) have studied the effect of vinblastine, a drug related to vincristine, on interferon production and found

little adverse influence; however, these authors noted this drug diminished interferon secretion.

St. Geme *et al.* (21) investigated the effects of 6MP on the ability of chick embryo fibroblasts to produce interferon, and while documenting resultant yields were slightly diminished, these authors felt the differences were not significant.

The discrepancy between the results of some of these studies and our own are multifaceted, but likely result from inherent dissimilarities between the animals used and between the basic cell types studied.

1. Levine A, Schimpf S, Graw R, Young R. Hematologic malignancies and other marrow "Failure States: Progress in the management of complicating infections. Semin Hematol 11:141–201, 1974.
2. Chabner B, Myers C, Olivera V. Clinical pharmacology of anticancer drugs. Semin Oncol 4:165–191, 1977.
3. Liddle G, Melmon K. The adrenals. In: Williams R, ed. Textbook of Endocrinology. Philadelphia, Saunders, pp233–322, 1974.
4. Loo T, Luce J, Sullivan S, Frei E. Clinical pharmacological observations on 6-mercaptopurine and 6-methylthiopurine ribonucleoside. Clin Pharmacol Ther 9:180–194, 1968.
5. Owellen R, Root M, Hains F. Pharmacokinetics of vindisine and vincristine in humans. Cancer Res 27:2603–2607, 1977.
6. Cesario T, Schryer P, Tilles J. The relationship between the physicochemical nature of human interferon, the cell induced and the inducing agent. Antimicrob Agents Chemother 11:291–298, 1977.
7. Tilles J, Finland M. Microassay for human and chick cell interferon. Appl Microbiol 16:1706–1707, 1968.
8. Tidd D, Paterson A. A biochemical mechanism for delayed cytotoxic reaction of 6-mercaptopurine. Cancer Res 34:738–746, 1974.
9. Heller E. Enhancement of Chikungunya virus replication and inhibition of interferon production by actinomycin D. Virology 21:652–656, 1963.
10. Wagner RR. The interferons: Cellular inhibitors of viral infection. Annu Rev Microbiol 17:285–297, 1963.
11. Wagner RR, Huang A. Reversible inhibition of interferon synthesis by puromycin. Evidence for an interferon specific messenger RNA. Proc Nat Acad Sci USA 54:1112–1115, 1965.
12. Calabresi P, Parks R. Chemotherapy of neoplastic diseases. In: Gilman A, Goodman L, Gilman L, eds. The Pharmacological Basis of Therapeutics. New York, MacMillan, 1980.
13. Kilbourne E, Smart K, Pokorney B. Inhibition by cortisone of the synthesis and action of interferon. Nature (London) 190:650–651, 1961.
14. Postic B, DeAngeles C, Breinig M, Hoe M. Effects of cortisol and adrenalectomy on induction of interferon by endotoxin. Proc Soc Exp Biol Med 125:89–92, 1967.
15. Talas M, Stoger I. Hormone pretreatment and interferon production in mice. Acta Virol 16:211–216, 1972.
16. DeSomer P, Billeau A, DeClercq E. Influence of N'-methylacetamide on interferon production in vivo in rats. In: Rita G, ed. Interferons. New York, Academic Press, pp65–81, 1968.
17. Mendelson J, Glasgow L. The in vitro and in vivo effects of cortisol on interferon production and action. J Immunol 96:345–362, 1966.
18. DeMaeyer E, DeMaeyer J. Two sided effect of steroids on interferon in tissue culture. Nature (London) 197:724–725, 1963.
19. Adolf G, Swetly P. Glucocorticoid hormones inhibit DNA synthesis and enhance interferon production in human lymphoid cell line. Nature (London) 282:736–738, 1979.
20. Havell EA, Vilcek J. Inhibition of interferon secretion by vinglastine. J Cell Biol 64:716–719, 1975.
21. St Geme J, Horrigan D, Toyama P. The effect of 6-mercaptopurine on the synthesis and action of interferon. Proc Soc Exp Biol Med 130:852–855, 1960.

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Immune Response to Laminin, a Noncollagenous Glycoprotein of Basement Membrane, in a Syngeneic Murine System[1] (41484)

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Abstract. The immune response to the noncollagenous, attachment glycoprotein of basement membrane (laminin) was studied in a syngeneic murine system. Delayed-type hypersensitivity (DTH) was assayed in C57BL/6 mice by measuring footpad swelling following challenge with connective tissue antigens. Mice receiving a single sensitizing injection of laminin in Freund's complete adjuvant (FCA) developed a significant DTH response which peaked on Day 7, 24 hr after challenge with laminin or collagenase-treated laminin. Laminin-sensitized mice failed to show any significant footpad swelling when challenged with types I or IV collagen, or fibronectin throughout these experiments. Normal mice displayed no significant DTH when challenged with these collagenous or noncollagenous connective tissue antigens. Adoptive transfer of laminin-sensitized spleen cells into normal mice resulted in significant DTH responsiveness to challenge with laminin; depletion of T cells from the immune spleens abrogated this response. Twenty-four hours after challenge with laminin, histology of the footpad lesions of laminin-sensitized mice revealed a mononuclear cell infiltrate, characteristic of a DTH response. Mice receiving repeated injections of laminin in Freund's incomplete adjuvant (FIA) developed significant antibody responses as detected by the enzyme-linked immunosorbent assay (ELISA). Furthermore Pronase, but not collagenase, treatment of laminin destroyed its antigenicity. Laminin immune sera showed no reactivity when assayed on fibronectin or collagen types I–V. No cross-reactivity was exhibited by murine anti-type IV or anti-type I collagen antisera with laminin. These studies demonstrate the ability of isologous laminin to induce antigen-specific cell-mediated and humoral immunity in a murine model.

Basement membranes are extracellular tissue structures which separate parenchymal cells from underlying connective tissue matrices. Basement membranes provide a supportive framework for epithelial (1, 2) and endothelial cells, and function as a barrier to the passage of macromolecules (3). Several basement membrane-specific proteins have been identified: type IV collagen (3), laminin (4), or GP-2 (5, 6), a heparan sulfate proteoglycan (7, 8) and entactin (9, 10).

Tissues containing a high concentration of basement membrane (i.e., lung and kidney) are frequent sites of immunologically mediated injury; however, until recently, specific immunity to basement membrane components has been primarily examined through the use of specific antibodies to aid in the characterization of its biochemical

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[3] Abbreviations used: ABTS, 2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate]; anti-Thy 1.2, monoclonal anti-mouse Thy 1.2 antibody; C, guinea pig complement; DTH, delayed-type hypersensitivity; EHS, Engelbroth–Holm/Swarm sarcoma; ELISA, enzyme-linked immunosorbent assay; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; PBS–Tween–BSA, PBS (pH 7.8) containing 0.05% Tween 20 and 1% bovine serum albumin; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; T cell, thymus-derived lymphocyte.

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properties and tissue distribution. We have previously demonstrated the ability of types IV and I collagen to induce cell-mediated and humoral immunity in a murine model (11–13). Antibodies to type IV collagen have been identified in the sera of patients with systemic sclerosis (scleroderma) (14). The levels of these anti-collagen autoantibodies correlated directly with the extent of interstitial lung disease in this patient population. These studies implicate type IV collagen as an autoantigen and immunity to this basement membrane protein may be involved in initiating and/or perpetuating this disease process.

Laminin, a disulfide-linked glycoprotein is present in all basement membranes in quantities approximately equal to type IV collagen (4). Although the role of laminin in the basement membrane has not been completely determined, it appears to function in cell attachment similar to fibronectin with type I collagen; however, laminin is biochemically and immunologically distinct from fibronectin (4, 15). Sakashita *et al.* (16) demonstrated that laminin specifically binds to heparin and heparan sulfate. They suggest that an interaction of laminin with heparan sulfate proteoglycans of the basement membrane may contribute to the structural integrity of basement membranes *in vivo*. In addition, Ekblom *et al.* (17) have suggested that laminin may be involved in the increased cell adhesiveness observed during the aggregation phase of kidney morphogenesis. In the present study, we have examined the ability of laminin, isolated from the Engelbroth–Holm/Swarm (EHS) sarcoma, to induce an immune response in a syngeneic murine system. Our data demonstrate cell-mediated immunity to laminin, following a single sensitizing exposure, and the development of a strong antibody response after repeated immunizations with this basement membrane antigen.

Materials and Methods. *Animals.* C57BL/6 female mice (Laboratory Animal Medicine, Medical University of South Carolina) were used at 6–8 weeks of age.

Antigens. Laminin was isolated from the EHS sarcoma as described by Timpl *et al.* (4). Tumors were homogenized in 3.4 *M* NaCl (0.05 *M* Tris–HCl, pH 7.4) at 4° to remove soluble extracellular proteins. The residue was extracted twice with 0.5 *M* NaCl (0.05 *M* Tris–HCl, pH 7.4) at 4°. All extractions contained the protease inhibitors phenylmethane sulfonyl fluoride (160 μg/ml; Sigma Chemical Co., St. Louis, Mo.) and *N*-ethylmaleimide (1.25 mg/ml; Sigma). Type IV collagen was removed from the 0.5 *M* NaCl extract by increasing the salt concentration to 1.7 *M* and centrifuging the solution at 10,000 rpm. The supernatant fluid containing laminin was dialyzed against 2 *M* urea (0.05 *M* Tris–HCl, pH 8.6) and passed over a diethylaminoethyl (DEAE) cellulose column equilibrated with the same buffer. The unbound material containing laminin was concentrated by ultrafiltration (Diaflo filter XM 100, Amicon Corp., Lexington, Mass.). The concentrated sample was chromatographed on an agarose A-1.5m column, equilibrated and eluted with 1 *M* $CaCl_2$ (0.05 *M* Tris–HCl, pH 7.4). Laminin appeared in the void volume and was lyophilized following dialysis against 0.05% acetic acid.

Collagen types I and IV were prepared as described previously (11, 18). Type I collagen was isolated from murine tail tendon. The EHS sarcoma served as the source of type IV collagen. Following pepsinization of the homogenized tumor tissue, the collagen was purified by sequential acetic acid solubilizations, precipitation with NaCl, and DEAE-cellulose chromatography. Human fibronectin was obtained from Collaborative Research Inc. (Waltham, Mass.).

Analysis of antigens. Laminin and collagen types I and IV were assayed for purity by amino acid analysis, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and susceptibility to protease-free bacterial collagenase. No evidence of other collagen types or non-collagenous impurities were observed in the collagen preparations as previously described (11). Following reduction with 2-mercaptoethanol, the laminin preparation exhibited two homogeneous bands on SDS–polyacrylamide gels (4%) with mo-

lecular weights slightly greater than 200,000 and 400,000. Treatment of laminin with protease-free bacterial collagenase prior to analysis by SDS–PAGE did not alter the gel patterns. Treatment of types I and IV collagens in the same manner eliminates their gel bands (11). Thus the laminin preparation appears free of collagenous contaminants.

Collagenase and Pronase treatments. Laminin was dissolved in 0.005 *M* $CaCl_2$ (0.05 *M* Tris–HCl, pH 7.6) at a concentration of 2.5 mg/ml. Bacterial collagenase (*Clostridium histolyticum;* Millipore Corp., Freehold, N.J.) was purified by the method of Peterkofsky and Diegelmann (19) and was free of noncollagenase proteolytic activity as we have previously demonstrated (11). Purified bacterial collagenase or Pronase (Calbiochem–Behring Corp., La Jolla, Calif.) was added at concentrations of 20 units/125 μl and 0.2 mg/125 μl, respectively. The samples were incubated at 37° for 18 hr, and subsequently dialyzed extensively against phosphate-buffered saline (PBS).

Delayed-type hypersensitivity. Five micrograms laminin in 0.1 ml of 0.1 *M* acetic acid was emulsified with an equal volume of Freund's complete adjuvant (FCA; Gibco, Grand Island, N.Y.) and injected subcutaneously (sc) in the abdomen; control mice were untreated. DTH was measured by footpad swelling in response to antigenic challenge as described previously (11). Briefly, mice were injected intradermally in the plantar surface of the hindfoot with 5 μg antigen in a volume of 0.03 ml. Footpad thickness was determined 4 and 24 hr postchallenge with a micrometer and compared to measurements observed prior to antigen injection. The data are expressed as the mean percentage footpad swelling ± standard error (SE):

$$\% \text{ footpad swelling} = \frac{\text{mm after challenge} - \text{mm before challenge}}{\text{mm before challenge}} \times 100.$$

Passive transfer. Spleens were removed from normal or laminin-sensitized mice 7 days after immunization. The sensitized spleen cells were left untreated or were incubated with monoclonal murine anti-Thy 1.2 antibody (Anti-Thy 1.2; New England Nuclear, Boston, Mass.) plus guinea pig complement (C; M.A. Bioproducts, Walkersville, Md.) as previously described (11). Twenty-five million untreated or T-cell-depleted laminin-sensitized spleen cells, or 25 × 10^6 normal spleen cells were injected intraperitoneally (ip) in 0.5 ml PBS into normal mice. Two days after cell transfer, recipient mice were challenged in the footpad with 5 μg laminin and footpad swelling was assayed 24 hr later.

Histology. Normal and laminin-sensitized mice were challenged in the footpad on Day 6 with 5 μg laminin. Twenty-four hours later, mice were sacrificed and their feet fixed in a neutral gluteraldehyde solution, followed by embedding in glycol methacrylate (Polysciences, Inc., Warrington, Pa.). Sections were stained with hematoxylin and eosin, and examined by light microscopy (11).

Preparation of antisera. Rabbit antibodies against types I and IV collagen were prepared as described previously (14). Rabbit anti-laminin serum was obtained using the same immunization protocol. A New Zealand white rabbit was injected in each hind footpad on Days 0 and 14 with 0.5 mg laminin in FCA. On Days 23 and 33, the animal was injected sc with 1 mg laminin in Freund's incomplete adjuvant (FIA; Gibco). Blood was collected from the ear on Days 30 and 40, and by cardiac puncture on Day 41. A gamma globulin fraction was obtained following ammonium sulfate precipitation (14).

Mice were immunized on Days 0, 14, 21, 28, and 35 by sc injections of 50 μg laminin, type IV collagen, or type I collagen dissolved in 0.1 *M* acetic acid and emulsified with an equal volume of FIA. Immune and normal mice were bled from the ophthalmic venous plexus on Day 42. The sera from each group (at least four mice per group) were pooled and stored at −70°.

Enzyme-linked immunosorbent assay (ELISA). Antibodies to laminin were detected using the ELISA as we have de-

scribed previously (13, 14). Serial dilutions of rabbit antibodies (1 mg/ml) and immune mouse sera in PBS (pH 7.8) containing 0.05% Tween 20 and 1% bovine serum albumin (PBS–Tween–BSA), were applied to microtiter wells (Flow Laboratories, McLean, Va.) coated with 1.25 μg laminin and incubated for 45 min. After washing, 100 μl of peroxidase-conjugated goat anti-rabbit immunoglobulin (IgG, M, A) antibodies (1:500, Cappel Laboratories, Cochranville, Pa.) or peroxidase-conjugated goat anti-mouse immunoglobulin (IgG, M, A) antibodies (1:250; Cappel) in PBS–Tween–BSA were added, the plates incubated for an additional 45 min, and then washed. One hundred microliters of the substrate, 0.03% ABTS (2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate], Sigma), was added to each well in 0.1 *M* citrate buffer (pH 4.0) with 0.05% H_2O_2, and after incubation for 1 hr, the absorbance was read at 414 nm on a Titertek Multiskan (Flow). All assays were performed in duplicate and the results are expressed as the mean absorbance value.

Absorption assays. Repeated absorptions of normal and immune mouse sera were performed to remove anti-laminin antibody activity. A 1:20 dilution of each serum was plated as described above. After a 45-min incubation, the sera were transferred to other laminin-coated wells. This process was continued for up to four absorptions, and the assay was completed by the addition of peroxidase-conjugated immunoglobulin and substrate. In order to control for the loss of volume resulting in reduction of the sera activity on laminin following each transfer, mouse anti-type I and anti-type IV collagen sera were plated on laminin. These sera were then transferred to

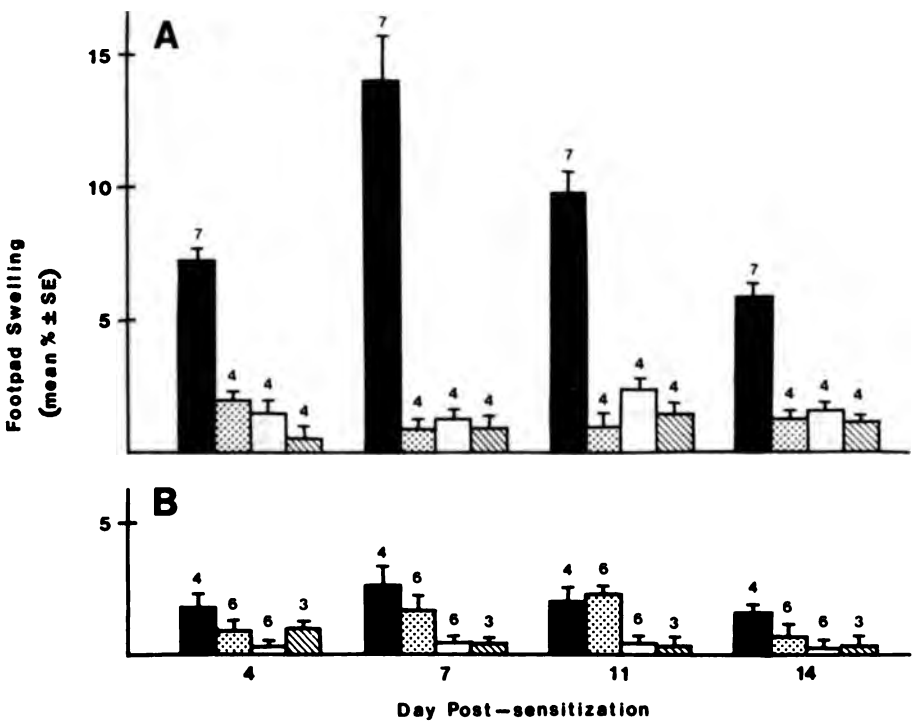

FIG. 1. DTH response of (A) mice sensitized with 5 μg laminin in FCA and (B) normal mice challenged on Days 3, 6, 10, and 13 postsensitization with laminin (■), type I collagen (▤), type IV collagen (□), and fibronectin (▨). DTH was assayed 24 hr postchallenge and the results are expressed as mean percentage footpad swelling ± SE. The number of mice assayed is indicated above each bar.

plates coated with their respective antigen. No significant loss of reactivity with the collagen antigens was observed by the anti-collagen sera after the four absorptions with laminin.

Results. The ability of C57BL/6 mice to mount a DTH response to homologous laminin is shown in Fig. 1. Groups of mice were immunized with 5 μg laminin in FCA on Day 0; normal controls were untreated. Mice were challenged with 5 μg laminin, type I collagen, type IV collagen, or fibronectin on Days 3, 6, 10, and 13 postsensitization. Footpad swelling was assayed 4 and 24 hr later, with maximal swelling occurring at 24 hr. No significant swelling was observed at 4 hr, indicating that the response was not due to an antibody-mediated Arthus reaction or to an immediate hypersensitivity response. As shown in Fig. 1A, mice sensitized and challenged with laminin demonstrated significant DTH on Day 4 (7.2% ± 0.5), with the response peaking 7 days postsensitization (13.8% ± 1.9). Laminin-sensitized mice showed no significant DTH when challenged with types I or IV collagen, or fibronectin. Normal control mice (Fig. 1B) failed to show significant swelling at any time when challenged with laminin, types I or IV collagen, or fibronectin.

To eliminate the possibility that a collagenous protein contaminant in our laminin preparation was contributing to the observed DTH response, mice were challenged with collagenase and Pronase-treated laminin (Table I). Normal and laminin-sensitized mice showed no significant footpad swelling on Day 7, 24 hr after challenge with Pronase-treated laminin (Table I, Expt 1). Challenge of these same mice with untreated laminin immediately following assay of the footpad swelling on Day 7 resulted in significant footpad swelling ($P < 0.025$) on Day 8 in the laminin-sensitized mice only. Laminin-sensitized mice challenged on Day 6 with collagenase-treated laminin displayed significant DTH 24 hr later; normal controls showed no significant swelling (Table I, Expt 2). These data indicate that noncollagenous protein antigens are responsible for the

TABLE I. COLLAGENASE AND PRONASE TREATMENT OF LAMININ USED FOR DTH CHALLENGE[a]

Experiment	Sensitization	No. of mice	Challenge 1	Footpad swelling (mean % ± SE) Day 7	Challenge 2	Footpad swelling (mean % ± SE) Day 8
1	None	3	Pronase-treated laminin	0.16 ± 0.13	Laminin	0.3 ± 0.30
	Laminin	4	Pronase-treated laminin	0.92 ± 0.46	Laminin	7.3 ± 0.50
2	None	3	Collagenase-treated laminin	0.94 ± 0.31		
	Laminin	4	Collagenase-treated laminin	10.4 ± 1.10		

[a] Mice were sensitized with 5 μg laminin in FCA; control mice were untreated. Mice were challenged on Day 6 with 5 μg Pronase or collagenase-treated laminin preparations. Footpad swelling was determined at 24 hr (Day 7). Mice in Experiment 1 were immediately challenged with untreated laminin following assay of footpad swelling on Day 7. Footpad swelling was determined in these groups after 24 hr (Day 8).

observed DTH response in the laminin-sensitized mice.

The ability of laminin-sensitized spleen cells to adoptively transfer DTH responsiveness to normal syngeneic mice is shown in Table II. Spleen cells were obtained from laminin-sensitized mice on Day 7, the peak of the DTH response (Fig. 1). Twenty-five million sensitized spleen cells were injected ip into normal syngeneic mice, which were challenged 48 hr later with laminin; these mice displayed significant footpad swelling 24 hr after challenge. Mice receiving an ip injection of 25×10^6 sensitized spleen cells which were pretreated with a monoclonal murine anti-Thy 1.2 antibody plus C failed to display significant footpad swelling when challenged with laminin. Injection of 25×10^6 normal C57BL/6 spleen cells into normal mice did not transfer reactivity to laminin. These data indicate that Thy 1.2-positive T lymphocytes are responsible for mediating the DTH response to laminin.

Histological examination of the footpad lesions of laminin-sensitized mice on Day 7, 24 hr after challenge with laminin, revealed an inflammatory response with a predominantly mononuclear cell infiltrate (Figs. 2A and B). Histologic sections obtained from the footpads of normal mice after challenge with laminin showed no significant inflammation (Figs. 2C and D). The identification of a mononuclear cell infiltrate in the footpad lesions of the laminin-immune mice is a feature characteristic of a DTH response.

The ability of C57BL/6 mice to elicit an antibody response to homologous laminin was examined using the ELISA. Rabbit antisera against laminin were included in all assays as positive controls and to determine reproducibility of the procedure. Rabbit antisera to types I or IV collagen display no significant reactivity when assayed on laminin-coated wells. No antibody was detected in the sera of C57BL/6 mice displaying DTH as a result of a single injection of laminin; however, sera obtained from mice receiving repeated sc injections of 50 μg laminin in FIA displayed significant antibody reactivity (laminin titers >320) when assayed on laminin (Fig. 3). Minimal background absorbance was exhibited by normal mouse sera at all dilutions and represent background values (Fig. 3). Sera obtained from mice sensitized to type I and type IV collagen using the same immunization schedule as with laminin displayed no significant reactivity when assayed on laminin (Fig. 3); however, these sera do exhibit high antibody responses when assayed on wells coated with their respective immunogens (13). The anti-laminin mouse sera did not react with wells coated with fibronectin or types I–V collagen (data not shown).

Treatment of the laminin-coated wells with purified bacterial collagenase (11) had no effect on the ability of the anti-laminin sera to react with laminin (Fig. 4A); Pronase treatment of laminin-coated wells eliminated the observed anti-laminin reactivity of the immune sera. Repeated absorptions of the anti-laminin sera on laminin-coated wells were performed to determine if all laminin-specific activity could be removed, since a single absorption did not remove all activity (data not shown). As shown in Fig. 4B, reactivity of laminin immune sera was reduced to background by sequential absorptions on laminin-coated wells. Thus, the antibody reactivities of these immune sera are spe-

TABLE II. ADOPTIVE TRANSFER OF DTH TO LAMININ USING UNTREATED AND T-CELL-DEPLETED SENSITIZED SPLEEN CELLS

Sensitization[a]	Cell treatment	Challenge	No. of mice	Footpad swelling (mean % ± SE)
None	None	Laminin	4	1.5 ± 0.38
Laminin	None	Laminin	6	9.7 ± 1.20
Laminin	Anti-Thy 1.2 + C	Laminin	8	1.3 ± 0.36

[a] Donor mice were either untreated or immunized with 5 μg laminin in FCA.

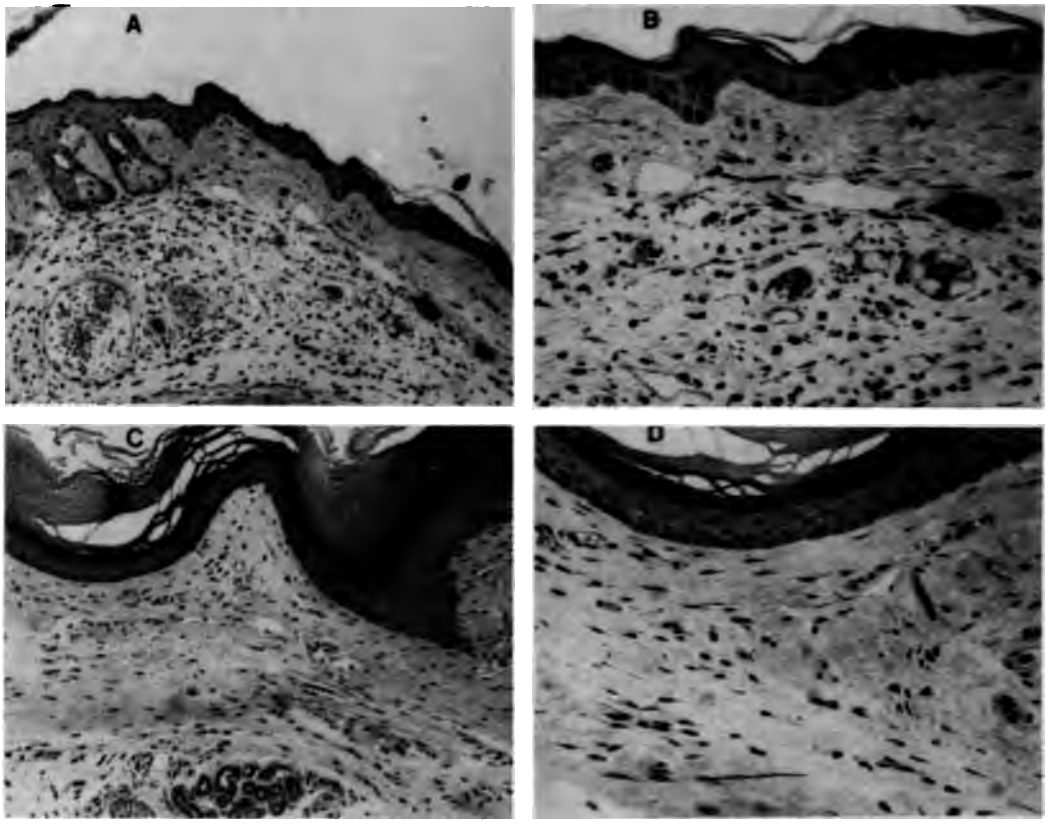

FIG. 2. Histological examination of the footpad lesions from laminin-sensitized and normal mice 7 days postsensitization and 24 hr after challenge with laminin. Sections from a laminin-sensitized mouse showing (A) an inflammatory response (×145) and (B) the presence of a mononuclear cell infiltrate (×285). Sections from a normal mouse challenged with laminin showing no inflammation (C, ×145; D, ×285).

cific for a noncollagenous protein of the basement membrane.

Discussion. Noncollagenous macromolecules are integral components of basement membranes interacting with type IV collagen to generate this extracellular matrix. Laminin, the major noncollagenous glycoprotein of basement membrane, has been shown by indirect immunofluorescence to be associated with a wide variety of mammalian tissues (4, 15) and, by electron microscopy, to be localized primarily in the lamina rara of the basement membrane (15, 20). Laminin, consisting of two polypeptide chains (220,000 and 440,000 daltons) linked by disulfide bonds, is biochemically and antigenically distinct from type IV collagen and fibronectin (4). The Engelbroth–Holm/Swarm (EHS) sarcoma is a transplantable murine tumor which produces an extracellular matrix of basement membrane components (21). The present studies were performed using the EHS tumor as the source of laminin and type IV collagen. Previous investigators have demonstrated that antigens expressed on type IV collagen and laminin isolated from the EHS tumor matrix cross-react with normal human and murine basement membranes (4, 15, 22, 23). C57BL/6 mice were used in the present studies for both passage of the EHS tumor line and as hosts for laminin sensitization.

The basis for cell-mediated immunity

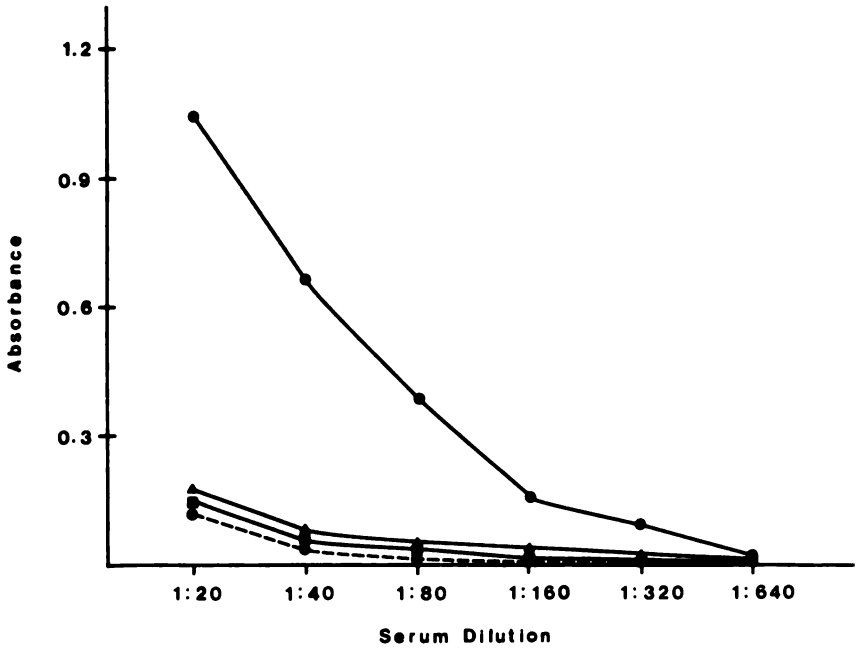

FIG. 3. Analyses of immune and normal mouse sera on laminin-coated wells using the ELISA. Serial dilutions of mouse anti-laminin sera (●), mouse anti-type I collagen sera (▲), mouse anti-type IV collagen sera (■), and normal mouse sera (– – –) were assayed on microtiter wells coated with 1.25 μg laminin. The results are expressed as the mean absorbance value at 414 nm.

(CMI) to connective tissue components was established earlier by Adelmann and coworkers (24, 25) and by Senyk and Michaeli (26). These investigators demonstrated that type I collagen could serve as an antigen for the induction of a delayed-type hypersensitivity (DTH) response; however, they reported conflicting results regarding the ability of an animal to mount an immune response to homologous collagens. Senyk and Michaeli (26) demonstrated DTH skin reactions in guinea pigs to homologous type I collagen. However, Adelmann *et al.* (24, 25) were unable to demonstrate CMI in guinea pigs to homologous type I collagen employing similar techniques. These observations may be attributable to the strain differences of experimental animals.

Our data demonstrate the ability of homologous laminin to induce cell-mediated and humoral immunity in a murine system. Mice receiving a single sc injection of 5 μg laminin in FCA developed significant footpad swelling 24 hr after challenge with laminin. This DTH response reached a maximal level on Day 7 postsensitization. The response was specific for laminin and was not observed after challenge with type I collagen, type IV collagen, or fibronectin. Histological examination of the footpad lesion revealed an infiltration of mononuclear cells, indicative of a classical DTH response. Laminin-sensitized spleen cells adoptively transferred DTH responsiveness to normal syngeneic mice and depletion of T cells by prior treatment of the immune spleen cells with anti-Thy 1.2 serum plus C eliminated the ability to transfer this response. Thus, mice sensitized to laminin mount an antigen-specific DTH response to this basement membrane antigen.

Although antibody was not detected in mice displaying DTH to laminin after a single injection, higher concentrations of the sensitizing antigen and use of protocols employing repeated immunization elicited significant antibody titers to laminin. No cross-reactivity was exhibited by antisera

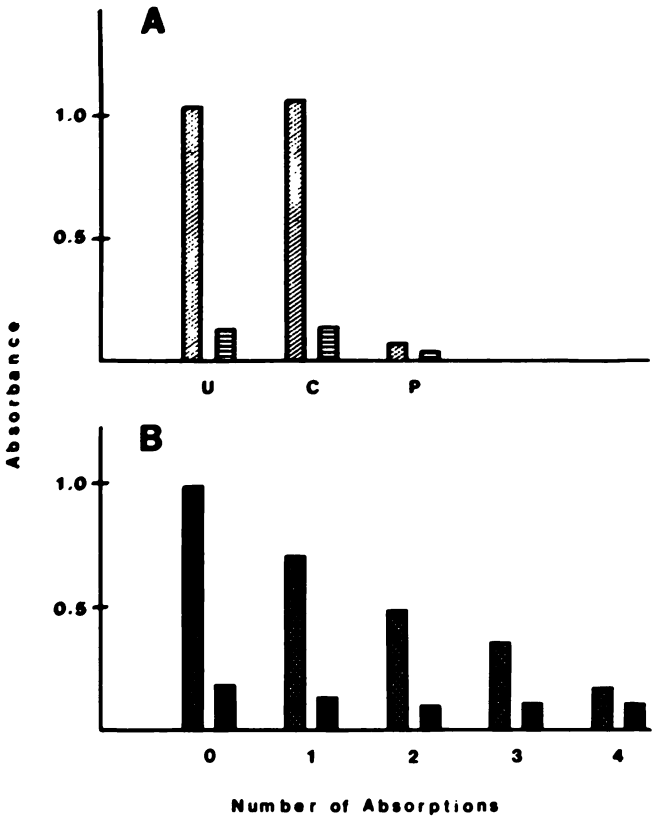

FIG. 4. **(A)** Reactivity of normal (▤) and anti-laminin (▥) mouse sera on microtiter wells coated with 1.25 μg (U) untreated laminin, (C) collagenase-treated laminin, and (P) Pronase-treated laminin preparations. **(B)** Repeated absorptions of anti-laminin (▨) and normal (■) mouse sera on laminin-coated wells. All sera were assayed at a 1:20 dilution on wells coated with 1.25 μg laminin. The results are expressed as indicated in Fig. 3.

to types I or IV collagen assayed with laminin, nor did laminin immune sera react with other connective tissue components. Collagenase treatment of laminin prior to analysis in the ELISA showed no alteration in reactivity of the sera with laminin.

Although the potential of basement membrane to act as an autoantigen has been shown, the precise antigens involved are in most instances unknown. Cell-mediated and/or humoral immunity to basement membranes could be directed at either collagenous or noncollagenous components, or both. Data from our laboratory support the hypothesis that basement membrane damage could be initiated or perpetuated by immunity to basement membrane collagen (13, 14). Significant titers of anti-type IV collagen antibodies were detected in the sera of some patients with scleroderma. Antibody levels correlated inversely with pulmonary diffusion capacity, a reliable early indicator of pulmonary interstitial disease. These anti-collagen autoantibodies were primarily of the IgM class, an immunoglobulin that is a potent activator of the complement cascade (13). Gay *et al.* (27) have reported autoantibodies to a collagenous component of basement membrane (C chain) in the sera of patients with epidermolysis bullosa simplex, a severe blistering skin disease. We have previously reported the induction of cell-mediated and humoral immunity to isologous type IV collagen in a

murine model (11, 13). Foidart *et al.* (28) demonstrated anti-laminin and anti-type IV procollagen antibodies in the sera of patients with Goodpasture's syndrome. Szarfman *et al.* (29) have reported that sera from humans with Chagas' disease and Rhesus monkeys infected with *Trypanosoma cruzi* contain antibodies which react with laminin. Furthermore, injection of affinity-purified sheep anti-laminin IgG into rabbits results in antibody binding to the glomerular basement membrane, with subsequent alterations in glomerular structure and proteinuria (30). These studies indicate that immunity to basement membrane components may be involved in disease processes.

Changes in vascular basement membranes occur during the progression of diseases such as scleroderma, atherosclerosis, and diabetes mellitus. Alterations in basement membrane structure and/or assembly may reflect endothelial damage, which may result in exposure of the basement membrane and induction of immunity to previously sequestered basement membrane components. Our observations and those of Foidart *et al.* (28) and Szarfman *et al.* (29) suggest that laminin, the major noncollagenous glycoprotein of basement membranes, is a strong immunogen, capable of inducing cell-mediated and humoral immunity. Studies are currently underway to investigate the possible immunopathological sequelae which may occur following the induction of immunity to homologous laminin in mice. The nature of the body components which elicit an immune response (autoimmunity) in connective tissue diseases is the subject of continuing investigation. The development of an animal model based on laminin immunity may lead to a better understanding of the inflammatory responses which occur in human and experimental animal diseases involving basement membranes.

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1. Terranova VP, Rohrbach DH, Martin GR. Role of laminin in the attachment of PAM 212 (epithelial) cells to basement membrane collagen. Cell 22:719–726, 1980.
2. Hogan B. Laminin and epithelial cell attachment. Nature (London) 290:737–738, 1981.
3. Kefalides NA. Structure and biosynthesis of basement membranes. Int Rev Connect Tissue Res 6:63–104, 1973.
4. Timpl R, Rhode H, Robey PG, Rennard SI, Foidart JM, Martin GR. Laminin—A glycoprotein from basement membranes. J Biol Chem 254:9933–9937, 1979.
5. Chung AE, Freeman IL, Braginski JE. A novel extracellular membrane elaborated by a mouse embryonal carcinoma-derived cell line. Biochem Biophys Res Commun 79:859–868, 1977.
6. Chung AE, Jaffe R, Freeman IL, Vergnes JP, Braginski JE, Carlin B. Properties of a basement membrane-related glycoprotein synthesized in culture by a mouse embryonal carcinoma-derived cell line. Cell 16:277–287, 1979.
7. Kanwar YS, Farquhar MG. Presence of heparan sulfate in the glomerular basement membrane. Proc Nat Acad Sci USA 76:1303–1307, 1979.
8. Hassell J, Robey PG, Barrach HJ, Wilczek J, Rennard SI, Martin GR. Isolation of a heparan sulfate-containing proteoglycan from basement membrane. Proc Nat Acad Sci USA 77:4494–4498, 1980.
9. Carlin B, Jaffe R, Freeman IL, Chung AE. Isolation and characterization of sulfated glycoproteins from a basement membrane related extracellular matrix. Fed Proc 39:1791, 1980.
10. Carlin B, Jaffe R, Bender B, Chung AE. Entactin, a novel basal lamina-associated sulfated glycoprotein. J Biol Chem 256:5209–5214, 1981.
11. Mackel AM, DeLustro F, LeRoy EC. Cell-mediated immunity to homologous basement membrane (type IV) collagen in C57BL/6 mice. Clin Immunol Immunopathol 21:204–216, 1981.
12. DeLustro F, Mackel AM, DeLustro B, LeRoy EC. Human monocyte regulation of connective tissue growth. Amer Zool, in press, 1982.
13. Mackel AM, DeLustro F, DeLustro B, Fudenberg HH, LeRoy EC. Immune response to connective tissue components of the basement membrane. Connect Tissue Res, in press, 1982.
14. Mackel AM, DeLustro F, Harper FE, LeRoy EC. Antibodies to collagen in scleroderma. Arthritis Rheum 25:522–531, 1982.
15. Foidart JM, Bere EW, Yaar M, Rennard SI, Guillino M, Martin GR, Katz SI. Distribution and immunoelectron microscopic localization of laminin, a noncollagenous basement membrane glycoprotein. Lab Invest 42:336–342, 1980.

16. Sakashita S, Engvall E, Ruoslahti E. Basement membrane glycoprotein laminin binds to heparin. FEBS Lett 116:243–246, 1980.
17. Ekblom P, Alitalo K, Vaheri A, Timpl R, Saxen L. Induction of a basement membrane glycoprotein in embryonic kidney: Possible role of laminin in morphogenesis. Proc Nat Acad Sci USA 77: 485–489, 1980.
18. Timpl R, Bruckner P, Fietzek P. Characterization of pepsin fragments of basement membrane collagen obtained from a mouse tumor. Eur J Biochem 95:255–263, 1979.
19. Peterkofsky B, Diegelmann R. Use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. Biochemistry 10:988–994, 1971.
20. Madri JA, Roll FJ, Furthmayr H, Foidart JM. Ultrastructural localization of fibronectin and laminin in the basement membrane of the murine kidney. J Cell Biol 86:682–687, 1980.
21. Orkin RW, Gehron P, McGoodwin EB, Martin GR, Valentine T, Swarm R. A murine tumor producing a matrix of basement membrane. J Exp Med 145:204–220, 1977.
22. Timpl R, Martin GR, Bruckner P, Wick G, Wiedeman H. Nature of the collagenous protein in a tumor basement membrane. Eur J Biochem 84:43–52, 1978.
23. Timpl R, Glanville RW, Wick G, Martin GR. Immunochemical study on basement membrane (type IV) collagen. Immunology 38:109–116, 1979.
24. Adelmann BC, Kirrane JA, Glynn LE. The structural basis of cell-mediated immunological reactions of collagen. Characteristics of cutaneous delayed-hypersensitivity reactions in specifically sensitized guinea-pigs. Immunology 23:723–738, 1972.
25. Adelmann BC, Kirrane J. The structural basis of cell-mediated immunological reactions of collagen. The species specificity of the cutaneous delayed hypersensitivity reaction. Immunology 25:123–130, 1973.
26. Senyk G, Michaeli D. Induction of cell-mediated immunity and tolerance to homologous collagen in guinea pigs: Demonstration of antigen-reactive cells for a self-antigen. J Immunol 111:1381–1388, 1973.
27. Gay S, Ward WQ, Gay RE, Miller EJ. Autoantibodies to basement membrane collagen: Epidermolysis bullosa simplex versus bullous pemphigoid. J Cutan Pathol 7:315–317, 1980.
28. Foidart J, Foidart J, Dubois C, Mahieu P. Anticorps diriges contre la laminine et le procollagene de type IV dans le syndrome de Goodpasture. Nephrologie 1:57–60, 1980.
29. Szarfman A, Terranova VP, Rennard SI, Foidart JM, Lima MDF, Scheinman JI, Martin GR. Antibodies to laminin in Chagas' disease. J Exp Med 155:1161–1171, 1982.
30. Abrahamson DR, Caulfield JP. Anti-laminin-induced pathology in the rat glomerulus. Fed Proc 41:1272, 1982.

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Factors Modifying DNA Synthesis by Lung Fibroblasts *in Vitro*[1] (41485)

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Abstract. DNA synthesis in guinea pig lung fibroblast cultures was shown to be stimulated by endotoxins, PGE_2, CTAP-III, CTAP-P_2, and insulin; indomethacin and cortisol partially reversed some of these effects. DNA synthesis in human lung fibroblasts was also markedly stimulated by CTAP-III, while the response to endotoxins was less striking.

Lung fibroblast cultures derived from the gas exchange component of pulmonary parenchyma provide a potentially useful *in vitro* model system for studying pulmonary connective tissue metabolism (1–4). This culture system permits one to study new synthesis of connective tissue by lung fibroblasts, and to study separately the replication of the cells and the chemical anatomy of the extracellular matrix materials secreted by pulmonary fibroblasts as these various activities may be modified by physiologic, pathologic, and pharmacologic agents.

In the present study, we focused on lung fibroblast proliferative responsiveness (measured by incorporation of [*methyl*-^{3}H]thymidine into fibroblast DNA) to relevant agonists of both exogenous and endogenous origin. Our interest in this aspect of pulmonary inflammation originates in the possibility that an expanding population of lung fibroblasts may be an important antecedent to accelerated fibrosis and deposition of extracellular matrix materials. Supporting this idea is evidence from experimental cotton pellet granulomas in rats showing a direct correlation between the amount of connective tissue DNA and the accumulating extracellular connective tissue matrix materials of the progressing inflammatory process (5). The inflammatory response in rat lung to intermittent oxygen exposure was also accompanied by elevated DNA values during the later "fibroblastic" phase of inflammation (6). Rabbit lung fibroblast cultures exposed to various dusts often responded with increased DNA synthesis in parallel with increased synthesis of extracellular matrix materials including collagen and glycosaminoglycans (7).

If fibroblast proliferation is important to mounting a destructive fibrotic process in pulmonary tissue, factors capable of accelerating or repressing lung fibroblast DNA synthesis may have practical significance. With this in mind, we have examined the effect of gram-negative endotoxins analogous to what might be released in lung during bacterial pneumonitis. Further, since immune complex-mediated injury to pulmonary endothelium results in local secretion of platelet-derived growth factors, we have examined the effect of two platelet-derived connective tissue activating peptides, CTAP-III (8, 9) and CTAP-P_2 (10). In addition, we examined the effects of prostaglandin E_2 (PGE_2), known to be secreted by lung fibroblasts (11).

Materials and Methods. *Culture methods.* Guinea pig lung fibroblasts were isolated by enzymatic disaggregation of lung parenchyma and plating at high density in plastic flasks as previously reported (1). Human lung fibroblast cultures were established in the same manner from normal tissue removed at lobectomy for neoplasm. Cultures derived in this way were propagated using medium F-12 (with 10% fetal calf serum, FCS 10%) or medium 1066, 90%: FCS 10%. Three different guinea pig lung fibroblast lines and one human lung fibroblast line were used in this study.

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Complete medium changes were carried out three times a week and trypsin dispersal was performed as required for propagation or study. Freezing procedures were as previously reported.

DNA synthesis was measured by determining the incorporation of [*methyl*-^{3}H]-thymidine into cells in microtiter well cultures utilizing 10^4 cells per culture (8).

Sources of mediators. CTAP-III was isolated from outdated human platelets by methods previously reported (8, 9). This cationic protein appears to be the major human platelet derived growth factor; it was essentially homogeneous in multiple PAGE systems and its sequence has been established (12). A minor platelet-derived growth factor, *CTAP-P*$_2$, is an anionic material which stimulates DNA and GAG synthesis in human synovial cells. *CTAP-P*$_2$ was partially purified as a by-product of the isolation of CTAP-III, i.e., from biologically active fractions which did not react with antisera to CTAP-III (10). The major contaminant identified in CTAP-P$_2$ preparations is albumin.

The enteric *endotoxins* were obtained from Difco Laboratory, Detroit, Michigan, while the *Klebsiella pneumoniae* LPS was a gift from Dr. A. I. Braude, University of California, San Diego. Crystalline bovine insulin was obtained from Sigma Chemical Company, St. Louis, Missouri, and polyinosinic:polycytidylic acid (Poly I:C) was a gift from Dr. A. A. Tytell, Ph.D., Merck Sharp and Dohme, West Point, Pennsylvania. Prostaglandin E$_2$ (PGE$_2$) was kindly provided by Dr. John E. Pike, The Upjohn Company, Kalamazoo, Michigan.

Results. *Endotoxin stimulation of DNA synthesis.* Bacterial endotoxin was shown to be capable of substantially stimulating the synthesis of DNA in lung fibroblast cultures (Table I). It is noteworthy that very small amounts of LPS were not uncommonly more stimulatory than higher concentrations. It is clear that among the potential inhibitors of DNA synthesis, that cycloheximide virtually ablated *basal synthesis*, indomethacin at clinically achievable concentrations was without effect, and cortisol exhibited marginal suppression of *basal* DNA synthesis.

It was noteworthy that both cortisol and indomethacin partially reversed the *stimulatory effect* of *E. coli* LPS on lung fibroblast DNA synthesis. The observations recorded for *E. coli*:026:B6 endotoxin was representative of the guinea pig lung fibroblast response to other endotoxins including: *S. typhosa*-0901, *E. coli*:0111:B4, *S. typhimurium*, and *S. minnesota*.

Of some interest was the observation (Tables I and II) that PGE$_2$, 1 μg/ml, signifi-

TABLE I. AGENTS MODIFYING ENDOTOXIN STIMULATION OF [^{3}H]DNA SYNTHESIS IN GUINEA PIG LUNG FIBROBLAST CULTURES[a]

Additives	[^{3}H]DNA (cpm/10^4 cells)[b]	Experimental/ control	*P*
0.15 *M* NaCl	3,787 ± 584	—	—
PBS[c]	4,271 ± 708	—	—
E. coli 026:B6, LPS, 1.0 μg/ml	24,380 ± 5935	6.4	<0.01
E. coli LPS, 50 μg/ml	14,045 ± 3684	3.7	<0.01
Cortisol, 1.0 μg/ml	2,781 ± 716	0.7	<0.05
Cycloheximide, 10 μg/ml	110 ± 17	0.03	<0.01
Indomethacin, 15 μg/ml	2,962 ± 1077	0.8	NS[d]
E. coli LPS, 50 μg + cortisol	4,799 ± 1980	1.3	<0.01
E. coli LPS, 50 μg + indomethacin	8,227 ± 1562	2.2	<0.02
E. coli LPS, 50 μg + cycloheximide	69 ± 21	0.02	<0.01
PGE$_2$, 1.0 μg/ml	7,032 ± 1859	1.9	<0.01

[a] Target cultures were GP-22 lung fibroblasts.
[b] Data are expressed as the mean ± 1 SD for four to six microcultures in this and subsequent tables.
[c] PBS refers to buffered saline containing 0.15 *M* NaCl plus 0.05 *M* phosphate buffer, pH 7.0.
[d] Not significant.

TABLE II. AGENTS MODIFYING PLATELET OR INSULIN STIMULATION OF [^{3}H]DNA SYNTHESIS IN GUINEA PIG LUNG FIBROBLAST CULTURES[a]

Additives	[^{3}H]DNA (cpm/10^4 cells)	Experimental/control	P
0.15 *M* NaCl	7,826 ± 1718	—	—
PBS	6,921 ± 1711	—	—
CTAP-III, 21 μg/ml	39,123 ± 2006	5.0	<0.01
CTAP-III + indomethacin	23,183 ± 97	3.0	<0.01
CTAP-III + cortisol	35,722 ± 2418	4.6	NS[b]
Cortisol, 1.0 μg/ml	10,405 ± 868	1.3	<0.05
Indomethacin, 15 μg/ml	7,175 ± 412	0.9	NS
CTAP-P_2, 163 μg/ml	15,793 ± 1615	2.0	<0.01
CTAP-P_2 + indomethacin	8,967 ± 1792	1.1	<0.01
CTAP-P_2 + cortisol	10,821 ± 2375	1.4	<0.05
Insulin, 0.5 unit/ml	40,450 ± 3516	5.2	<0.01
Insulin, 5.0 unit/ml	42,258 ± 4311	5.4	<0.01
Insulin, 5 unit/ml + indomethacin	28,016 ± 973	3.6	<0.05
Insulin, 5 unit/ml + cortisol	47,519 ± 1364	6.1	NS
E. coli LPS, 026:B6, 50 μg/ml	11,263 ± 339	1.6	<0.02
PGE_2, 1.0 μg/ml	17,705 ± 1376	2.3	<0.01
PGE_2, 5 μg/ml	11,545 ± 1366	1.5	<0.01

[a] The GP-20 lung fibroblast line served as target cultures in this experiment.
[b] Not significant.

cantly stimulated DNA synthesis. Not all experiments demonstrated PGE_2 stimulation of DNA synthesis; on occasion, very high concentrations (5 μg/ml) were inhibitory while 0.1 μg/ml was stimulatory.

Stimulation of DNA synthesis by platelet factors and insulin. It was clear that both platelet factors, CTAP-III and CTAP-P_2, stimulated DNA synthesis in guinea pig lung fibroblast cultures to an extent comparable to that in the endotoxin experiments (Table II). In the present experiment, the specific stimulation by CTAP-III seemed to be greater than that generated by CTAP-P_2, although the less purified state of the CTAP-P_2 makes such calculations imprecise. Indomethacin significantly reduced the stimulatory effect of CTAP-III while cortisol was apparently ineffective. Both indomethacin and cortisol significantly reversed the stimulatory effect of the minor platelet factor, CTAP-P_2.

Bovine insulin in pharmacologic concentrations was shown to be a potent stimulator of DNA synthesis in these cell cultures, an effect which was resistant to cortisol suppression. On the other hand, indomethacin was able to partially suppress the insulin-stimulated increase in DNA synthesis.

Stimulation of DNA synthesis in human lung fibroblast cultures. Lung fibroblast cultures of human origin showed a marked increase in DNA synthesis on exposure to the major platelet mitogen CTAP-III, and relatively minor levels of stimulation by the synthetic polynucleotide, Poly I:C, (Table III).

Insulin caused a minor increase in DNA synthesis, cycloheximide ablated DNA synthesis, and cortisol exhibited a borderline stimulatory effect. The enteric endotoxins had no significant effect on DNA synthesis; *K. pneumoniae* LPS caused a significant, but minor, stimulation of lung fibroblast DNA synthesis. PGE_2 (data not shown) did not stimulate DNA synthesis in the human lung fibroblast cultures.

Discussion. In general, endotoxin stimulation of DNA synthesis was greater in guinea pig lung fibroblast cultures than in our human line. Earlier studies demonstrated that the lipid A core of endotoxins was a potent stimulator of glycosaminoglycan synthesis in human synovial cultures (13), and recently lung fibroblasts were

TABLE III. STIMULATION OF [^{3}H]DNA SYNTHESIS BY HUMAN LUNG FIBROBLASTS

Mediator or vehicle	[^{3}H]DNA (cpm/10^4 cells)	Experimental/ control	*P*
Experiment 1			
0.15 *M* NaCl	1,074 ± 105	—	—
CTAP-III, 25 μg/ml	11,069 ± 4532	10.3	<0.01
Poly I:C, 50 μg/ml	2,243 ± 414	2.1	<0.01
Experiment 2			
0.15 *M* NaCl	11,417 ± 1060	—	—
CTAP-III, 19 μg/ml	35,636 ± 2076	3.12	<0.01
Insulin, 0.4 μg/ml[a]	16,669 ± 1634	1.46	<0.01
Cortisol, 1.0 μg/ml	14,181 ± 1197	1.24	<0.05
Cycloheximide, 10 μg/ml	1,944 ± 273	0.17	<0.01
E. coli 026:B6 endotoxin 0.5 μg/ml	12,962 ± 1736	1.14	NS
S. tymphimurium endotoxin, 5.0 μg/ml	12,943 ± 1579	1.22	NS
Klebsiella endotoxin, 0.5 μg/ml	14,240 ± 733	1.25	<0.01

[a] 0.4 μg corresponds to 10 munit. Normal human insulin values range from 4 to 10 μunit/ml of serum.

shown to exhibit increased GAG synthesis on exposure to endotoxins (14). Whether this relative resistance of our human lung fibroblast line to endotoxin stimulation of DNA synthesis is a peculiarity of the cell line or representative of such cells remains to be established.

These data demonstrate that CTAP-III, a growth factor derived from human platelets, markedly stimulates DNA synthesis in lung fibroblast cultures of guinea pig and human origin. Since CTAP-III is secreted by platelets in the course of the aggregation-granule release process (8, 15), it is likely that relatively large concentrations of this mediator flood local areas of injury. While it is possible that CTAP-P_2 is also presented to injured tissue in the same manner, there is yet no unambiguous data on its secretion mechanism. One may speculate that an injurious process in lung, be it physical, chemical, immunologic, or microbiologic, may initiate fibroblastic proliferation in part through this mechanism. In guinea pig lung cultures, it was possible to partly suppress this response with the anti-inflammatory agent indomethacin in "clinically relevant" concentrations.

1. Castor CW, Heiss PR, Gray RH, Seidman JC. Connective tissue formation by lung cells *in vitro*. Amer Rev Resp Dis 120:107–119, 1979.
2. Castor CW, Wilson SM, Heiss PR, Seidman JC. Activation of lung connective tissue cells *in vitro*. Amer Rev Resp Dis 120:101–106, 1979.
3. Seidman JC, Castor CW. Connective tissue activation in guinea pig lung fibroblast cultures: Regulatory effects of glucocorticoids. In Vitro 17:133–138, 1981.
4. Castor CW, Fremuth TD. Lung fibroblast metabolism: Factors regulating glycosaminoglycan synthesis. Clin Res 28:752A, 1980.
5. Castor CW. Connective tissue activation. V. The flux of connective tissue activating peptide during acute inflammation. J Lab Clin Med 81:95–104, 1973.
6. Välimäki M, Juva K, Rantanen J, Ekfors T, Niinikoski J. Collagen metabolism in rat lungs during chronic intermittent exposure to oxygen. Aviat Space Environ Med 46:684–690, 1975.
7. Hext PM, Richards RJ. Biochemical effects of asbestiform minerals on lung fibroblast cultures. Brit J Exp Pathol 57:281–285, 1976.
8. Castor CW, Ritchie JC, Scott ME, Whitney SL. Connective tissue activation. XI. Stimulation of glycosaminoglycan and DNA formation by a platelet factor. Arthritis Rheum 20:859–868, 1977.
9. Castor CW, Ritchie JC, Williams CH, Scott ME, Whitney SL, Myers SL, Sloan TB, Anderson B.

Connective tissue activation. XIV. Composition and actions of a human platelet autacoid mediator. Arthritis Rheum 22:260–272, 1979.

10. Castor CW, Cobel-Geard SR. Connective tissue activation: Evidence for a second human platelet growth factor. Clin Res 28:139A, 1980.
11. Bryant RW, Feinmark SJ, Makheja AN, Bailey JM. Lipid metabolism in cultured cells. Synthesis of vasoactive thromboxane A_2 from [^{14}C] arachidonic acid by cultured lung fibroblasts. J Biol Chem 253:8134–8142, 1978.
12. Walz DA, Castor CW. Connective tissue activation: Structural studies on a human platelet mitogen. Clin Res 27:649A, 1979.
13. Buckingham RB, Castor CW. The effect of bacterial products on synovial fibroblast function: Hypermetabolic changes induced by endotoxin. J Clin Invest 51:1186–1194, 1972.
14. Castor CW, Fremuth TD, Furlong AM. Lung fibroblast metabolism: Characterization of proteoglycan (PG) and glycosaminoglycan (GAG) synthesis. Clin Res 29:738A, 1981.
15. Castor CW, Cobel-Geard SR, Hossler PA, Kelch RP. Connective tissue activating peptide-III. XXII. A platelet growth factor in human growth hormone deficient patients. J Clin Endocrinol Metab 52:128–132, 1981.

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Spontaneous Production of High Levels of Leukocyte (α) Interferon by a Human Lymphoblastoid B-Cell Line (LDV/7) (41486)

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Abstract. A human lymphoblastoid B-cell line (LDV/7) capable of replicating significant levels of transfer factor and immune RNA was found to produce high quantities of interferon continuously. The maximum yield of interferon (600 units/ml) was detected on the seventh day. The interferon was characterized as human leukocyte (α) type. This interferon exhibited broad antiviral activity against vesicular stomatitis virus, poliovirus, sindbis virus, *Herpesvirus saimiri*, and Epstein–Barr virus. Treatment of LDV/7 cells with TPA, PHA, or LPS failed to enhance interferon production. Furthermore, LDV/7 cells could not be induced with Sendai virus to produce larger amounts of interferon. Interferon production correlated best with cell proliferation. It is therefore possible that this cell line may be a good source for large-scale production of human leukocyte interferon as well as for cloning of the interferon genome, and for studies of gene expression.

A human lymphoblastoid cell line (LDV/7), established in 1968 from the peripheral blood leukocytes of a healthy 65-year-old male, has been shown to be the only lymphoblastoid cell line capable of replicating significant quantities of transfer factor (TF) and immune RNA (1, 2). Other human lymphoblastoid cell lines (B or T), or cell lines established from Burkitt's lymphoma (3, 4), including Namalwa (3), were found to be unsatisfactory for replication of TF. Since LDV/7 is a B-cell line, possessing a unique ability to replicate TF, we were interested: (a) in investigating its ability to produce interferon spontaneously or by induction with Sendai virus or other viruses, as has been reported previously for other lymphoblastoid cells (5–8), and (b) in testing the antiviral activity of this interferon, particularly on two primate oncogenic herpesviruses, Epstein–Barr virus (EBV) of man and *Herpesvirus saimiri* (HVS) of the squirrel monkey.

Materials and Methods. The characterization of LDV/7 cells, including surface markers, cytochemistry, immunoglobulin secretion, HLA type, chromosome analysis, presence of EBV DNA and antigens, and induction of tumors in athymic nude mice has been reported elsewhere (9). LDV/7 cells were grown at 37° in RPMI–1640 medium (Gibco) containing 10% heat-inactivated fetal bovine serum and antibiotics. Unlike some B-cell lines, LDV/7 cells are unique in their growth pattern since more than 15% of the cells firmly attach to the plastic surface, and clumps of cells, both attached and floating, are consistently present in culture.

Treatment of cells for interferon production. LDV/7 cells were seeded at 2×10^5 cells/ml in RPMI 1640 medium containing 10% fetal calf serum. At the time of seeding >95% cells were viable. These cells were divided into various groups, as described below, for treatment with the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and Sendai virus. Supernatants were harvested at various times and were clarified at 300*g*, filtered through a 0.45-μm filter and frozen in liquid nitrogen. The procedure was as follows:

1. LDV/7 cells supernatants harvested at 3, 7, 10, and 14 days;

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2. LDV/7 cells treated with 20 ng/ml of TPA for 3, 7, and 14 days and supernatants harvested as for untreated LDV/7 cells;

3. LDV/7 cells infected with 4 HA unit of Sendai virus and supernatants harvested as for TPA-treated cells;

4. LDV/7 cells treated with phytohemagglutinin (PHA-P), 2 μg/ml and *Escherichia coli* lipopolysaccharides (LPS), 25 μg/ml; and

5. LDV/7 cells treated first with PHA-P (2 μg/ml) and then with 20 ng/ml of TPA.

Assays for interferon. The assays were performed by the VSV plaque reduction method as described previously (10, 11). The titers are expressed as international reference units per milliliter.

EBV and HVS sensitivity to LDV/7-produced interferon. This was carried out according to the methods previously described (12). The Ag-876 strain of EBV from cells grown at 34° was used for induction of EBV capsid antigens (VCA) and transformation of human cord blood lymphocytes (HCBL) (13). The titer of this virus had previously been shown to be 10^6 transforming units/ml in HCBL.

Results. *Growth characteristics of LDV/7 cells.* LDV/7 cells form clumps consisting of 5–400 cells. These clumps either attach to the plastic surface or float in the culture medium. On subculture, the floating cells and/or attached cells were found to attach to the plastic surface as well as float in the medium. LDV/7 cells replicate faster than Raji, Ag-876, P3HR-1 cells, and Namalwa (6).

Interferon production. The spontaneous levels of inhibitor detected in the supernatants of LDV/7 cells at various times are presented in Fig. 1. The peak of inhibitor production (approximately 600 units) was on the seventh day and thereafter the level of interferon declined.

The LDV/7 cells treated with TPA showed only slight enhancement of inhibitor production by 0.2_{10} (Fig. 1) on Day 7; however, on Day 3 approximately 500 units of interferon were detected in TPA-treated cells (an increase of 380 units). On Day 14, TPA-treated samples showed an increase of only 30 units over the spontaneously produced inhibitor.

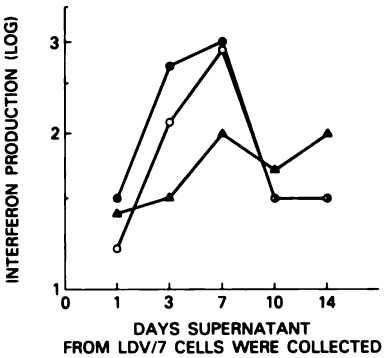

FIG. 1. Detection of interferon (log) in LDV/7 lymphoblastoid cell cultures in the presence and absence of TPA and Sendai virus. ○, LDV/7 supernatant only; ●, LDV/7 supernatant with the presence of TPA; ▲, LDV/7 supernatant harvested after infection with Sendai virus.

The Sendai virus infected LDV/7 cells showed a significant drop in the level of the inhibitor (Fig. 1) indicating that LDV/7 cells contain a considerable level of inhibitor used to neutralize the virus.

LDV/7 cells treated with 2 μg/ml of PHA-P or 25 μg/ml of LPS for 3 and 7 days failed to induce any increase in the level of spontaneously produced inhibitor (≤120 units Day 3 and ≤400 units Day 7). However, the treatment of LDV/7 cells first with PHA and then with TPA resulted in producing ≥600 units of the inhibitor, but this effect decreased significantly by Day 7 (≤160 units).

Characterization of the inhibitor produced by LDV/7 cells. As presented in Table I, the inhibitor was characterized as being interferon. The inhibitor showed the following major properties of human leukocyte interferon: (a) the inhibitor was active against different viruses: sindbis virus, poliovirus, and vesicular stomatitis virus; (b) the inhibitor was species specific in that it protected human WISH and human Hep-2 cells but it did not protect mouse L cells against virus infection; (c) the inhibitor was stable to acid treatment (pH 2) at 4° for 24 hr; (d) the antiviral activity was cell mediated since the washing of the cells treated for 24 hr with the inhibitor did not remove the resistance to virus infection; (e) the inhibitor was inactivated by antiserum

TABLE I. CHARACTERIZATION OF VIRUS INHIBITOR[a]

Sample treatment	Cell type:	Human WISH	Human WISH	Human Hep-2	Mouse L	pH 2 Human WISH
	Virus:	VS	Sindbis	Polio	VS	VS
LDV/7 supernatant harvested Day 3		120	>100	>100	—	100
LDV/7 supernatant harvested Day 7		600	>100	>100	<3	100
LDV/7 cells treated with 20 ng/ml of TPA supernatant, harvested Day 3		500	>100	>100	—	—
LDV/7 cells treated with 20 ng/ml of TPA supernatant, harvested Day 7		1000	>100	>100	<3	100
LDV/7 cells infected with 4 HA units of Sendai virus, supernatant harvested Day 7		500	>100	>100	—	—

[a] Data are expressed in international reference units/ml.
Inhibitor characteristics: (1) Broad antiviral activity; (2) cell species specificity; (3) stable at pH 2; (4) the inhibitor is cell associated since the inhibitor is not removed after three washings of the cells before virus challenge; (5) the inhibitor was destroyed by trypsin and not by DNase or RNase (data not shown); (6) the inhibitor was not sedimented by ultracentrifugation.

directed against leukocyte interferon (Table II); and (f) the inhibitor was destroyed by trypsin but not by DNase or RNase and was not sedimented by ultracentrifugation.

Antiviral activity. Results of LDV/7 lymphoid cell interferon on oncogenic HVS of nonhuman primates and EBV are presented in Table III. Continuous post-treatment of owl monkey kidney (OMK) cells with LDV/7 supernatant harvested Day 7 reduced the HVS titers by 3 logs. If the OMK cells were pretreated with the interferon, a further half-log reduction in HVS titer was observed.

The interferon from LDV/7 cells failed to induce any effects on intrinsic EBV genome expression in Raji cells, since no reduction or inhibition of spontaneous expression of EBV nuclear antigen (EBNA) was observed. This interferon also failed to block VCA-EA expression in EB virus producers Ag-876 or P3HR-1 cells (Table III). Superinfection of Raji cells with lytic P3HR-1 EBV normally leads to expression of early antigens (EA). Table III shows that after superinfection of Raji cells with $10^{4.0}$ EA inducing units, approximately 10% EA were detected by indirect IF. More than

TABLE II. ANTIGENIC TYPING OF LDV/7 INTERFERON[a]

Interferon sample	Medium	Antiserum: Anti-fibroblast (β) IFN	Antiserum: Anti-leukocyte (α) IFN
From LDV/7 cells	25	25	<10
Control fibroblast (β)	65	<1	65
Control leukocyte (α)	6	6	<1
Control immune (γ)	25	25	25

[a] The interferon titers are expressed after mixing the LDV/7 and control interferons with antiserum to fibroblast and leukocyte interferons.

TABLE III. INHIBITION OF HERPESVIRUS SAIMIRI-INDUCED CPE AND EBV EA AND TRANSFORMATION IN THE PRESENCE OF SUPERNATANT COLLECTED FROM LDV/7 CELLS, AFTER 7 DAYS OF INCUBATION

Cells/treatment/virus	Inhibition	
	In the presence of LDV/7 supernatant	In the absence of LDV/7 supernatant
1. (a) OMK cells were infected with serial dilutions of HVS with $10^{6.5}$/ml TCID50 titer for 2 hr, cells were washed and then continuously kept at 37° in maintainance medium with and without LDV/7 supernatant for 14 days and observed for HVS-induced CPE	$10^{3.5}$/ml	$10^{6.5}$/ml
(b) Pretreatment of OMK cells with LDV/7 spnt. for 24 hr at 37° before HVS infection	$10^{3.5}$ to $10^{3.0}$/ml	$10^{6.5}$/ml
2. Raji cells were infected $10^{4.0}$ EA inducing units/ml with P3HR1 EBV for 90 min. Then the cells were washed and fed with LDV/7 supernatant for 72 hr	≤1.0% cells expressing EA and no VCA	≥10% cells expressing EA and 3% VCA
3. Ag-876 cells growing at 34° were subcultured at seeding density of ≥1 × 10^6/ml and were fed with medium with or without LDV/7 supernatant for 7 days	10–15% EA/VCA	10–15% EA/VCA
4. HCBL separated by plasmagel were pretreated with LDV/7 supernatant (containing 600 units of interferon) at 37° for 24 hr. The other set of cord cells were kept without treatment with LDV/7 supernatant. The supernatant was removed and cells were washed with medium, infected with Ag-876 EBV with a titer of $10^{6.0}$/ml TFU, and observed for EBNA and transformation	$10^{5.0}$	$10^{6.0}$

90% EA were inhibited when cells were kept for 72 hr in supernatant collected from 7 day-incubated cells. HCBL pretreated for 24 hr with LDV/7 supernatant containing 600 U/ml of interferon were infected with various dilutions of transforming Ag-876 EBV. The data in Table III show that up to 10 transforming units of virus were inhibited, based on EBNA induction, colonies of transformed cells, and outgrowth of these cells under soft agar.

Discussion. The spontaneous production of interferon from other human lymphoblastoid cell lines has previously been reported (6, 7, 14). Pickering *et al.* (7) showed that with the exception of two B-cell lines (8876-7 and LukII) all other cell lines (Raji, CAH, Namalwa, BM, NBRL-AG57) either produced small amounts of interferon (10–200 units) or lacked interferon production (<10). Similarly Strander *et al.* (6) showed that the majority of the cell lines they tested produced <20 units of interferon; however, cell lines such as Namalwa, Akuba, P3HR-1, and Ly-46 could be induced to produce between 200 and 600 units of interferon. Thus LDV/7 cells may be included in the group of cells which spontaneously produce high amounts of interferon. Even though Namalwa cells can be used to produce higher yields of interferon with Sendai virus or treatment with

Poly(I)·poly(C) plus DEAE-dextran in the presence or absence of butyrate (5), the amount of interferon produced is comparable to that spontaneously produced by LDV/7 or 8866-7 and LuKII cells (300–1000 units). It seems therefore that LDV/7 cells, capable of replicating high levels of transfer factor and immune RNA, could be valuable in obtaining high yields of interferon in large-scale production without the costs and the complexity of the use of an inducer. In addition LDV/7 cells may be used for cloning the interferon gene and for studies of gene expression.

Our experiments in Fig. 1 showed that TPA, PHA-P, and LPS failed to increase the levels of interferon.

The absence of increased interferon production after mitogen stimulation may be due to interferon inhibition of mitogenic responses in LDV/7 cells. Szigeti *et al.* (15) showed that human leukocyte interferon at a concentration of 100 U/ml suppressed the mitogen (PHA)-induced lymphocyte migration. They further showed that if the lymphocytes were pretreated with interferon prior to PHA activation, it significantly reduced the action of leukocyte migration inhibitory factor. Thus the lack of increase in interferon levels in the LDV/7 supernatants in the presence of PHA or LPS may be due to the fact that LDV/7 cells are already proliferating cells and cannot be stimulated by PHA or LPS treatment. However, the action of PHA on stimulation of interferon production by T lymphocytes may be different, from the response of B cells like LDV/7 as suggested by Nathan *et al.* (14).

The kinetics of spontaneous interferon production by cell cultures has not been fully elucidated. The data of Pickering *et al.* (7) indicate that culture growth was found to correlate with the levels of interferon in cell lines spontaneously producing between 300 and 1000 units. They based this finding on spontaneously produced interferon being elaborated continuously in seeded cells into fresh medium at early saturation of density and harvesting samples of medium after incubation of 1 or 2 days. The data in Fig. 1 also indicate that interferon production in LDV/7 cells is correlated with cell growth. Levels of interferon from Days 1, 3, and 7 were increasing and the samples from Days 10 and 14 showed that a plateau of spontaneously produced interferon was reached. Since the LDV/7 cells grow very rapidly (doubling time 24 hr) the cell saturation density occurs between 6 and 7 days and immediately after the cells are in the lag phase. Thus these data further indicate that interferon was produced only during cell growth.

The test of crude interferon against two oncogenic herpesvirus of primates indicate that it is active against HVS and EBV, supporting our previous findings where interferon obtained from both leukocyte and lymphoblastoid cells was more effective than fibroblast interferon (12). Even though P3HR-1 and Ag-876 expressing EBNA and EA/VCA, and Raji cells expressing only EBNA were not affected by the treatment with the LDV/7 interferon, these virus-infected cells could be destroyed by effector cells activated by interferon (16, 17). Since the inhibition of transformation of cord blood cells by Ag-876 EBV was observed in low multiplicity of infection, perhaps more purified and higher doses of interferon may prevent transformation even with higher levels of viruses. On the other hand, the possibility of Ag-876 being less sensitive to interferon cannot be overlooked.

The effectiveness of the interferon produced by the LDV/7 cells *in vitro* against HVS and EBV provides promising support for *in vivo* trials against EBV- and HVS-induced tumors in subhuman primates.

The possibility that this interferon has properties distinct from those of virus-induced lymphoblastoid interferon should be studied. Attempts will be made to increase the levels of interferon produced, either by examining clones of LDV/7 cells or by using other stimulating agents.

1. Pizza G, Viza D, Boucheix C, Corrado F. In vitro production of a transfer factor specific for the cell carcinoma of the bladder. Brit J Cancer 33:601–611, 1976.

2. Viza D, Boucheix C, Phillips J, Cesarini JP, Phillips TM, Lewis MG, Pilch Y. Replication de l'ARN-immune par des cellules lymphoblastoides humaines. C R Acad Sci Paris 284:2565–2568, 1977.
3. Klein G, Dombos L, Gothoskar B. Sensitivity of Epstein–Barr virus (EBV) producer and nonproducer human lymphoblastoid cell line to superinfection with EBvirus. Int J Cancer 10:44–57, 1972.
4. Pizza G, Viza D, Boucheix C, Corrado F. Effect of in vitro produced transfer factor on immune responses of cancer patients. Eur J Cancer 13:917–924, 1977.
5. Johnston MD. Enhanced production of interferon from human lymphoblastoid (Namalwa) cells pretreated with sodium butyrate. J Gen Virol 50:191–194, 1980.
6. Strander H, Mogensen KE, Kantell K. Production of human lymphoblastoid interferon. J Clin Microbiol 1:116–117, 1975.
7. Pickering LA, Kronenberg LH, Stewart W. Spontaneous production of human interferon. Proc Nat Acad Sci USA 77:5938–5942, 1980.
8. Christofinis GJ, Steel CM, Finter NB. Interferon production by human lymphoblastoid cell lines of different origins. J Gen Virol 52:169–171, 1981.
9. Viza D, Boucheix C, Cesarini JP, Ablashi DV, Armstrong G, Levine P, Pizza G. Characterization of a human lymphoblastoid cell line, LDV/7, used to replicate transfer factor and immune RNA. Biology of the Cell, 1982, in press.
10. Green JA. Rapid assay of interferon. Texas Report on Biology and Medicine. 35:167–172, 1977.
11. Baron S, Dianzani F. General considerations of the interferon system. Tex Rep Biol Med 35:1–10, 1977.
12. Lvovsky E, Levine PH, Fuccillo D, Ablashi DV, Bengali ZH, Armstrong GR, Levy HB. Epstein–Barr virus and *Herpesvirus saimiri:* Sensitivity to interferon and interferon inducers. J Nat Cancer Inst 66:1013–1019, 1981.
13. Pizzo PA, Magrath IT, Chattopadhyay SK, Biggar RJ and Gerber P. A new tumor-derived transforming strain of Epstein–Barr virus. Nature (London) 272:629–631, 1978.
14. Nathan I, Groopman JE, Quan SG, Bercsh N, Golde DW. Immune (γ) interferon produced by a human T lymphoblastoid cell line. Nature (London) 292:842–844, 1981.
15. Szigety R, Masucci MG, Klein E, Klein G. Interferon suppresses antigen and mitogen induced leukocyte migration inhibition. Nature (London) 288:594–596, 1980.
16. Adams A, Strander H, Cantell K. Sensitivity of the Epstein–Barr virus transformed human lymphoid cell lines to interferon. J Gen Virol 38: 207–217, 1975.
17. Milfenhaus J, Damm M, Johannsen R. Sensitivity of various human lymphoblastoid cells to antiviral and anticellular activity of human leukocyte interferon. Arch Virol 54:271–277, 1977.

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Exercise and Estrus Cycle Influences on the Plasma Triglycerides of Female Rats (41487)

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Abstract. Since exercise and estrogens have a significant influence on plasma triglyceride (TG) concentration, this study was performed to determine the effects of exercise on the TG titers of female rats in the four stages of the estrus cycle. Normal female rats in the various phases of the estrus cycle, ovariectomized females, ovariectomized rats receiving estradiol, and normal male rats, all of comparable age, were run to exhaustion. At the time of exhaustion, the runner and a weight-matched control were anesthetized and exsanguinated. Ovariectomized animals receiving estrogen replacement ran 61% longer than the male rats. However, this difference probably resulted from body weight differences, because when positive work was calculated, all group means were equivalent. Resting plasma TG levels were higher in normal male rats than in any other group. Ovariectomy had no effect on plasma TG levels but estrogen administration increased the concentration by 35%. Phase of the estrus cycle had no effect on resting TG levels. Exercise reduced plasma TG levels in all groups. The exercise-induced plasma TG response was not influenced by the phase of the estrus cycle. The concentration of TG at exhaustion was equivalent for all groups regardless of the preexercise TG level. These findings suggest that, during exercise, animals with high resting TG titers divert a greater portion of this fuel to oxidation than to tissue TG synthesis.

Plasma triacylglycerols (TG) supply a portion of the fuel used by tissue for energy production (1). The muscle work of exercise increases energy utilization and fuel demand. It is well documented that exercise has a lowering effect on plasma TG. One bout of exercise will significantly lower the plasma TG level in humans (2) and rats (3).

The ovarian steroid hormone estrogen has a striking, while paradoxical, influence on the level of TG in the blood. Removal of estrogenic hormones by ovariectomy or menopause is associated with an elevation in plasma TG titers (4). On the other hand, females receiving ovarian steroid hormones in the form of birth control pills have higher TG levels than females having normal menstrual cycle levels of estrogens (5). Moreover, rats receiving estrogen injections also have circulating TG titers elevated above control levels (6). Since exercise and ovarian steroid hormones have a significant influence on plasma TG levels, it was the purpose of this study to determine the influence of an exhaustive bout of treadmill running on the plasma TG levels in female rats having altered circulating levels of estrogens.

Methods. Normal female, ovariectomized female, and male Sprague-Dawley rats (Harlan–Sprague-Dawley, Madison, Wisc.) were used in this study. At the time of sacrifice all animals were 4 months of age. The mean body weight of each group is given in Table I. Since animals were age matched, it can be seen that there were mean differences in body weight with males being heavier than any other group. All animals were housed individually and provided unrestricted access to Purina rat chow and water. The animal quarters were maintained at 23 ± 1° with a 12-hr light–dark cycle (0700–1900 hr light).

To determine the influence of removal of ovarian hormones, as well as the effect of estrogen replacement in ovariectomized rats on the parameters measured, surgical ovariectomy took place when the female animals were 3 months old. This was performed by removing the ovaries through a dorsal midline incision. Ovariectomy was characterized by a very small (67.4 ± 8.5 mg) uterine weight at sacrifice when com-

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TABLE I. BODY WEIGHTS OF NORMAL FEMALES, NORMAL MALES, OVARIECTOMIZED SHAM-INJECTED (OSI) FEMALES, AND OVARIECTOMIZED ESTROGEN-INJECTED (OEI) RATS

Normal females (64)	OSI females (12)	OEI females (12)	Normal males (20)
221 ± 2.6	248† ± 4.4	216 ± 3.1	308* ± 2.7

Note. All values are mean ± SEM; number of animals per group is given in parentheses.
* Significantly heavier than all other groups ($P < 0.05$).
† Significantly heavier than normal females and OEI females ($P < 0.05$).

pared to normal control female uteri (140 ± 8.2 mg). The mean body weight of these animals was also greater than normal. The day following surgery, 13 of the ovariectomized rats began receiving daily intramuscular hindlimb injections of 5 μg of estradiol-3 benzoate (Sigma Chemical Co.) in 0.2 ml of sesame oil (OEI group). The other 12 ovariectomized animals received daily 0.2 ml sham injections of sesame oil (OSI group). The mean uterine weight of the estrogen-injected animals was 314 ± 14 mg at the time of sacrifice.

All animals were indoctrinated to the treadmill with three 10-min running bouts. Only female animals were used that had normal estrus cycles, as determined by microscopic evaluation of vaginal smears (7). This procedure was also utilized as an indirect index of the circulating ovarian hormone titers. Since the effect of exercise on the plasma TG levels in males has been reported previously (3), we included a group of age-matched males in this study as a positive control.

On the day of the exhaustive treadmill run, animals were weighed and vaginal smears were evaluated to determine their stage of the estrus cycle. Animals found to be in the same stage of the estrus cycle were paired by weight and assigned to either the runner or control group. Exercised animals were run on a Quinton 42-15 rodent treadmill at 27.34 m/min up an 8% grade until the animals could no longer avoid the shocker. When the runner was removed from the treadmill, both runner and weight-matched control were ether anesthetized. The mean time of sacrifice of each group was similar to negate the circadian rhythm effect on the data. Rats were exsanguinated by cannulation of the abdominal aorta. Blood was collected in heparinized tubes and centrifuged. Plasma was stored at −80° until subsequent analysis for triglyceride concentration. Preliminary experiments indicated that freezing had no effect on the plasma TG values. The method of Fletcher (8) was used to determine the amount of TG in each sample. The value obtained for each animal was the mean of duplicate analysis performed on two separate occasions.

All values presented are group means ± SEM. Comparison of three or more means was performed using one-way analysis of variance. If an F ratio of $P < 0.05$ was calculated, a Dunnett's post hoc test was performed (9). Two means were compared using Student's t test.

Results. The mean run times of normal female rats at each phase of the estrus cycle, OSI females, OEI females, and normal males are given in Table II. The run time of the ovariectomized rats receiving estrogen replacement was significantly greater than mean run times obtained for the male and normal females. When the phase of the estrus cycle was considered, the OEI run time was significantly greater than mean values obtained for runners in the estrus and proestrus phases of the estrus cycle.

The total amount of positive work performed for each animal was calculated from body weight, speed, and run time (up an 8% incline). The mean value for each group is found in Table III. It can be seen that no significant differences existed between group means.

Mean plasma TG concentrations for each control and exercise group are given in

TABLE II. RUN TIMES TO EXHAUSTION FOR NORMAL FEMALE RATS AT EACH PHASE OF THE ESTRUS CYCLE, OVARIECTOMIZED SHAM-INJECTED (OSI) FEMALES, OVARIECTOMIZED ESTROGEN-INJECTED (OEI) FEMALES, AND NORMAL MALES

	Run times (min)
Normal females	
Estrus (10)	125.6 ± 8.7
Metestrus (11)	144.3 ± 9.9
Diestrus (10)	135.5 ± 16.7
Proestrus (6)	98.8 ± 18.0
Composite (37) (all normal female runners)	129.5 ± 6.8
Ovariectomized females	
OSI (6)	141.5 ± 16.2
OEI (6)	177.8 ± 21.3*
Males (10)	110.3 ± 12.6

Note. All values are means ± SEM; number of animals per group given in parentheses.
* Significantly greater than the run times for all the normal males, and all the normal females in the estrus and proestrus phases of estrus cycle.

TABLE III. POSITIVE WORK[a] PERFORMED FOR NORMAL FEMALE RATS AT EACH PHASE OF THE ESTRUS CYCLE, OVARIECTOMIZED SHAM-INJECTED (OSI) FEMALES, OVARIECTOMIZED ESTROGEN-INJECTED (OEI) FEMALES, AND NORMAL MALES

	Positive work performed (kg·m)
Normal females	
Estrus (10)	60.6 ± 4.4
Metestrus (11)	69.4 ± 4.8
Diestrus (10)	68.5 ± 9.5
Proestrus (6)	46.7 ± 7.6
Composite (37)	63.1 ± 3.4
Ovariectomized females	
OSI (6)	74.9 ± 9.1
OEI (6)	85.6 ± 2.0
Males (10)	74.3 ± 9.0

Note. All values are means ± SEM; the number of animals per group given in parentheses.
[a] Positive work was calculated from the product of: body weight (kg) × speed (m/min) × run time (min) × grade (0.08).

Table IV. When group control values were compared, results indicated that the ovariectomized female rats receiving estrogen had significantly higher concentrations of plasma TG than sham-injected ovariectomized rats, as well as the overall composite TG value for all the normal female rats. Male control plasma TG levels were significantly higher than all other control groups with the exception of the ovariectomized females receiving estrogen. Exercise produced a significant reduction in the plasma TG levels of all groups except the females in the diestrus stage of the estrus cycle.

The decrease in plasma TG as a result of treadmill running was determined by calculating the difference in plasma TG levels between each control and weight-matched

TABLE IV. PLASMA TRIGLYCERIDE CONCENTRATIONS FOR CONTROL AND EXHAUSTED MALE RATS, NORMAL FEMALE RATS, OVARIECTOMIZED FEMALE RATS (OSI), AND OVARIECTOMIZED FEMALE RATS RECEIVING ESTROGEN INJECTIONS (OEI)

	Plasma triglyceride (mg%)	
	Controls	Runners
Normal females		
Estrus	81 ± 8.87 (7)†·§	49 ± 2.5 (6)*
Metestrus	71 ± 7.8 (8)†·§	47 ± 3.7 (9)*
Diestrus	93 ± 7.7 (8)§	69 ± 12.7 (8)
Proestrus	93 ± 9.7 (6)§	53 ± 7.1 (7)*
Composite (All normal females)	84 ± 4.3 (29)†·§	55 ± 4.2 (30)*
Ovariectomized females		
OSI	92 ± 10.4 (6)†·§	51 ± 6.6 (6)*
OEI	124 ± 16.7 (6)	63 ± 5.7 (6)*
Males	129 ± 6.6 (10)	64 ± 7.0 (10)*

Note. All values are means ± SEM; number of animals per group given in parentheses.
* Significantly reduced plasma TG level compared to corresponding control value ($P < 0.05$).
† Significantly different from plasma TG level of OEI controls ($P < 0.05$).
§ Significantly different from plasma TG level of male controls ($P < 0.05$).

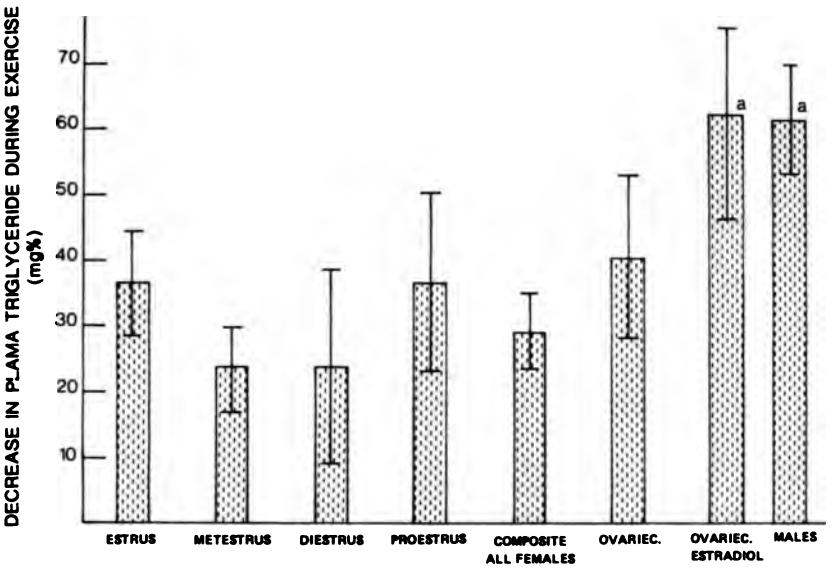

FIG. 1. The mean difference ± SEM of plasma triglyceride concentrations between runners and weight-matched controls is given for the following groups: males, ovariectomized females, ovariectomized females receiving estrogen, female rats in the various phases of the estrus cycle, and a composite value representing all normal female rats. (a) denotes a significant difference between the mean for the composite females and the group indicated.

runner. These mean values for each group are presented in Fig. 1. The TG reduction in estrogen-injected ovariectomized animals was 61.6 ± 15.5 mg%, a reduction of 49%. This exercise-induced decrease is similar to that calculated for the males of 48% (61.9 ± 8.8 mg% reduction). These decreases are significantly greater than the 20.4 mg% (35%) reduction computed for all the normal females.

Discussion. In the female rat, ovarian steroid hormones are responsible for the estrus cycle. The fluctuations that occur in estrogens and progesterone during this 4- to 5-day period are thought to be responsible for various rhythms seen in female mammalian physiology and biochemistry. Although the most pronounced influences are on uterine tissue, estrogenic rhythms have been demonstrated in body temperature (10), food intake (11), body weight (12), and voluntary running activity (13, 14). It has also been suggested that ovarian rhythms contribute to changes in blood hemoglobin content (15), plasma triglyceride titers (16), and tissue glycogen levels (17).

Since most exercise-related research avoids ovarian cycling as an experimental variable by using males or by ignoring cycling altogether, we have attempted to determine the effect of the ovarian estrus cycle on the plasma TG response to exhaustive treadmill running. The mean exercise time of ovariectomized female rats receiving estrogen was significantly greater than the means of all other groups. These findings are consistent with reports (13, 14) indicating that elevated ovarian estrogen titers increased voluntary running activity. Unfortunately, the relationship between voluntary running activity and run time to exhaustion is not known. These two parameters may not be related at all, since we found that stage of the estrus cycle had no effect on running endurance capacity. It would seem more reasonable to conclude that the increased run time in estrogen-treated rats was the result of group differences in body weight. When total positive work was estimated, taking body weight into consideration, work performance was equivalent for all groups.

It is evident from these data that the sex difference in plasma TG reported for humans (18), is also found in rats (19). The plasma TG level for male rats is approximately 50% higher than that determined in all the normal cycling females.

Estrogens have been shown to play a role in the metabolism of lipid (20, 21). However, no effect of phase of the estrus cycle on plasma TG was evident in this study. These results support the work of Punnonen (23) who found no effect of the menstrual cycle on plasma lipids. Unfortunately, Punnonen only studied three time points in the 28-day cycle. Two studies have reported that fluctuations in human TG (23) and low-density lipoproteins (24) exist during the menstrual cycle. However, differences in these parameters were not statistically significant.

While ovariectomy had no effect on plasma TG titers, daily injection of 5 μg of estradiol into ovariectomized rats significantly elevated the circulating lipid titers. Hamosh *et al.* (6) have shown that ovariectomy in rats had no effect on TG levels. This is in contrast to the works of others (25, 26), who report that prolonged removal of ovarian hormones, due to menopause or oophorectomy in humans, resulted in elevations in the concentration of plasma TG.

Ovarian hormone therapy has been used as a method of birth control. Numerous investigators, using both humans (27, 28) and experimental animals (16, 29), have reported estrogen-induced increases in plasma TG levels. We have confirmed this finding, since TG levels were elevated 37% by estrogen administration to ovariectomized rats.

The acute effect of exercise on the circulating level of TG in both man (30) and rat (3) is well documented. Recently, Terjung *et al.* (31) have shown that the uptake of plasma TG-derived fatty acids was increased in working skeletal muscle. This was supported by the results obtained in this study. Regardless of the experimental treatment, plasma TG levels were reduced as a result of one exhausting treadmill run. Phase of the estrus cycle did not quantitatively alter this exercise-induced reduction. It is interesting to note, however, that even though the initial level of TG in the plasma differed between groups, the level reached at exhaustion for all groups was approximately the same 56 mg%, a level similar to that reported by Reitman *et al.* (3) for animals that swam to exhaustion. Since all groups did approximately the same amount of work (Table III), these data (Fig. 1) suggest that significantly more energy may be derived from plasma TG in the working male and OEI female rats, than in the OSI and normal cycling females.

We wish to thank Mrs. Mary Ann Fritsch for her assistance in the preparation of this manuscript. This work was partially funded by a grant from the UIC Research Board.

1. Jones NL, Havel RJ. Metabolism of free fatty acids and chylomicron TG in the rat. Amer J Physiol 213:824–828, 1967.
2. Oscai LB, Peterson JA, Bogard DL, Beck RJ, Rothermel BL. Normalization of serum triglycerides and lipoprotein electrophoretic patterns by exercise. Amer J Cardiol 30:775–780, 1972.
3. Reitmann J, Baldwin KM, Holloszy JO. Intramuscular triglyceride utilization by red, white, and intermediate skeletal muscle and heart during exhaustive exercise. Proc Soc Exp Biol Med 142:628–631, 1973.
4. Shibata H, Matsuzaki T, Hatano S. Relationship of relevant factors of atherosclerosis to menopause in Japanese women. Amer J Epidemiol 109:420–424, 1979.
5. Carlton M, Simons LA, Gibson JC. Vascular disease risk factors in women with premature menopause. Horm Metab Res 12:208–211, 1980.
6. Hamosh M, Hamosh P. The effect of estrogens on the lipoprotein lipase activity of rat adipose tissue. J Clin Invest 55:1132–1135, 1975.
7. Turner CD, Bagnara JT. General Endocrinology, 6th ed. Philadelphia, Saunders, pp 456–468, 1976.
8. Fletcher MJ. A colorimetric method for estimating serum triglyceride. Clin Chim Acta 22:393–397, 1969.
9. Steel RGD, Torrie JH. Principles and Procedures of Statistics. New York, McGraw–Hill, 1960.
10. Abramson M, Torghele JR. Weight, temperature changes, and psychosomatic symtomology in relation to the menstrual cycle. Amer J Obstet Gynecol 81:223–232, 1961.
11. Attah MY, Besch EL. Estrus cycle variations of food and water intake in rats in the heart. J Appl Physiol 42:874–877, 1977.

12. Landau T, Zucker I. Estrogenic regulation of body weight in the female rat. Horm Behav 7:29–39, 1976.
13. Asdell SA, Doornenbal H, Sperling GA. Sex steroid hormones and voluntary exercise in rats. J Reprod Fertil 3:26–32, 1962.
14. Furusawa K. Some evidence of hormonal control in activity of skeletal musculature in man. Rev Canad Biol 21:325–334, 1962.
15. Vellar OD. Changes in hemoglobin concentration and hematocrit during the menstrual cycle. Acta Obstet Gynecol Scand 53:242–246, 1974.
16. Kim HJ, Kalkhoff RK. Sex steroid influence on triglyceride metabolism. J Clin Invest 56:888–896, 1975.
17. Palmer WK, Goldfarb AH, Ivy JL. Circadian and ovarian influences on tissue glycogen in female rats. Endocrinology 105:1254–1261, 1979.
18. Hagerup L, Hansen PF, Skov F. Serum cholesterol, serum triglyceride and ABO blood groups in a population of 50 year old Danish men and women. Amer J Epidemiol 95:99–103, 1972.
19. Bruckdorfer KR, Kang SS, Yudkin J. The hyperlipidemic effect of sucrose in male and female rats. Proc Nutr Soc 31:1119–1120, 1972.
20. Gerschberg H, Hulse M, Javier Z. Hypertriglyceridemia during treatment with estrogen and oral contraceptives. Obstet Gynecol 31:186–189, 1968.
21. Meade TW, Haines AP, North WRS, Chakrabarti R, Horwarth PJ, Stirling Y. Haemostatic, lipid, and blood-pressure profiles of women on oral contraceptives containing 50 μg or 30 μg oestrogen. Lancet 2:948–951, 1977.
22. Punnonen R. Total serum cholesterol triglycerides and phospholipids during the normal menstrual cycle. Int J Gynaecol Obstet 15:296–298, 1978.
23. Kim H-J, Kalkhoff RK. Changes in lipoprotein composition during the menstrual cycle. Metabolism 28:663–668, 1979.
24. Barclay M, Barclay RK, Skipski VP, Terebus-Kekish O, Mueller CH, Shah E, Elkins WL. Fluctuations in human serum lipoproteins during the normal menstrual cycle. Biochem J 96:205–209, 1965.
25. Hallberg L, Svanborg A. Cholesterol, phospholipids, and triglycerides in 50 year old women. Acta Med Scand 181:185–194, 1967.
26. Johansson BW, Kay L, Kullander S, Lenner HC, Svanborg L, Astedt B. On some late effects of bilateral oophorectomy, in the age range 15–30 years. Acta Obstet Gynecol Scand 54:449–461, 1965.
27. Corredor DG, Mendelsohn LV, Sabeh G, Sunder JH, Danowski TS. Serum lipids during oral contraceptive exposure. Clin Pharmacol Ther 111:188–193, 1970.
28. Hagopian M, Robinson RW. Effects of ethynylestradiol plus ethynylestradiol-3-methyl ether on serum triglyceride levels. Circulation 32 (Suppl. 2):14, 1967.
29. Schilling E, Gerhards E. Influence of hormonal contraceptives on carbohydrate and lipid metabolism in the rat. Biochem Pharmacol 22:1835–1844, 1973.
30. Gyntelberg F, Brennan R, Holloszy JO, Schonfeld G, Rennie MJ, Weidman SW. Plasma triglyceride lowering by exercise despite increased food intake in patients with type IV hyperlipoproteinemia. Amer J Clin Nutr 30:716–720, 1977.
31. Terjung RL, Budhoski L, Nazar K, Kobryn A, Kaciuba-Uscilko H. Chylomicron triglyceride metabolism in resting and exercising fed dogs. J Appl Physiol: Resp Environ Exercise Physiol 52:815–820, 1982.

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gram	g	nanometer	nm
hour	hr	normal (concentration)	*N*
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inside diameter	i.d.	ounce	oz
intramuscular	im	outside diameter	o.d.
intraperitoneal	ip	parts per million	ppm
intravenous	iv	percent	%
kilocalorie	kcal	picogram	pg
kilogram	kg	revolutions per minute	rpm
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The Antigen Receptor of Thymus-Derived Lymphocytes: Progress in the Characterization of an Elusive Molecule (41488)

JOHN J. MARCHALONIS[1] AND JEFFREY C. HUNT

Department of Biochemistry, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29425

Abstract. Thymus-derived lymphocytes (T cells) show remarkable specificity in their capacity to recognize non-self antigens and this recognition must serve as the initial step in the differentiation of immunologically competent T cells into antigen-specific effector cells including helpers, suppressors, and cytotoxic lymphocytes. The problem of determining the molecular nature of the receptor for antigen on these cells is a challenging area of investigation, and considerable insight into the serological and molecular properties of this receptor has recently been obtained using antibodies directed against immunoglobulin combining site regions as probes for the detection and isolation of the T-cell molecules. This review stresses results obtained within the past 3 years and (1) addresses the expression of immunoglobulin variable region determinants on T-cell receptors and factors, (2) presents a serological and molecular comparison of the structure of T-cell antigen-specific regulatory factors with those of receptors, and (3) presents a theoretical discussion of the genetics of antigen-specific T-cell factors and receptors. A pattern is emerging which indicates that T-cell receptors and some factors have a combining site which is related to immunoglobulin heavy chain variable regions. These molecules apparently do not bear determinants specified by the major histocompatibility complex (MHC), but express Ig-related variable regions and constant regions unique to T-cell products. The genes encoding these antigen-specific molecules (receptors, helper and suppressor factors) apparently are associated with the immunoglobulin heavy chain gene cluster. The intact V_H-related T-cell molecules have a subunit mass of approximately 68,000 daltons and can form disulfide-bonded dimers. Studies using proteolytic enzymes, coupled with antigenic and functional analyses, indicate that the molecule is composed of domains resembling those of immunoglobulin heavy chains, although the T-cell molecule does not bear classical heavy chain isotypic determinants. The formation of active suppressor or helper factors often requires association of V_H-related molecules with MHC-encoded proteins.

Thymus-derived lymphocytes (T cells) exhibit an exquisite specificity in their capacity to recognize antigen, and this recognition must serve as the initial step in the differentiation of immunologically competent T cells into antigen-specific effector cells such as helpers, suppressors, and cytotoxic lymphocytes. The problem of determining the molecular nature of the surface receptor for antigen on T cells is a challenging area of investigation and has been one of the major unresolved issues in modern immunology. It has been possible to gain considerable insight into the serological and molecular properties of this receptor using antibodies directed against combining site regions of immunoglobulins as probes for its detection, and for its isolation using immune affinity chromatography of T-cell receptor molecules. Antigen-specific T-cell immunoregulatory molecules such as helper and suppressor factors also have been shown to express determinants serologically related to variable regions of immunoglobulin heavy chains. A number of comprehensive reviews have been written regarding the problem of the T-cell receptor for antigen (1–6). In this brief review, we will focus predominantly upon data generated in the past 3 years and consider the following major issues regarding the T-cell receptor for antigen: First, the evidence supporting the existence of T-cell products

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related to immunoglobulin variable regions will be reviewed. Second, the relationship between T-cell receptors and T-cell released effector factors will be analyzed. Third, recent data regarding the characterization of isolated receptors and factors will be reviewed. Finally, based upon the molecular characterization data presently available, we will describe tentative models for the polypeptide structure of the T-cell receptor which is related to immunoglobulin heavy chain variable regions, and discuss possible models for the arrangement of genes encoding variable and constant regions of T-cell receptors and their relationships to immunoglobulin heavy chain V and C genes.

Existence of T-Cell Receptors and Factors Serologically Related to Immunoglobulin Variable Regions. Although a number of studies performed in the early 1970s indicated that some antisera directed against immunoglobulin determinants reacted with T-cell products and could be used in the isolation of immunoglobulin-related T-cell surface markers (6–11) and factors (12, 13), the location of these determinants on the immunoglobulin-related molecule was not established. In retrospect, it would appear that the original anti-immunoglobulin sera that were used to isolate immunoglobulin-related T-cell products must have done so because of a cross-reaction with variable region determinants because the ability of such sera to recognize determinants on T-cell molecules was not related to class-specific markers (6). Furthermore, subsequent studies established that such antisera were reactive with determinants lying in the Fd fragment of purified heavy chains (15), and also were associated with either interaction determinants formed by combination of V_H and V_L (16) or with restricted variable region framework determinants (17). Two types of antisera have been very useful in establishing a relationship between serological properties of the T-cell receptor and those of the combining site region of antibodies. The first type was the production of antibodies directed against idiotypic determinants on specific antibodies; the second type of reagent consisted of antisera directed against V_H framework determinants or to "non-idiotypic V_H-related determinants" which are localized to some presently unspecified area of the variable region of heavy chains. Table I gives a partial listing of characterized idiotypic determinants which have been detected either on the surfaces of T cells or an antigen-specific T-cell factors. *More than 13 defined idiotypic determinants have been found to be shared between antibodies and T cells or T-cell products exhibiting corresponding specificity.* A range of idiotypic specificities have been detected which include (a) specificity for defined low-molecular-weight haptens such as NP and the arsonate hapten, (b) specificities directed against naturally occurring proteins such as hen egg-white lysozyme, (c) specificities directed against synthetic polypeptides such as GAT and (T,G)A—L, (d) specificities for polysaccharides, and (e) specificities directed against MHC alloantigens in mice and rodents. Moreover, idiotypes cross-reactive with those of human myeloma immunoglobulins have been detected on the surface of certain peripheral T cells in man and on isolated idiotype-bearing receptors (40, 41). The question whether or not the idiotype-bearing surface molecules or factors detected were synthesized by the T cells has been answered affirmatively by a number of approaches (3, 4, 28, 30), and recent studies involving the production of T-cell hybridomas *in vitro* also strengthens the conclusion that T cells can synthesize and express idiotype related molecules (22, 31, 42, 43). The range of defined specificities and idiotypes of T cells suggests that, first, the recognition repertoire of antigen-specific T cells is diverse, and, second, that the antigen-specific products of T cells are serologically related in their combining site region to that of antibodies of corresponding specificities. It might be argued that the second property could arise by chance; that is, all proteins which bind a defined ligand with a certain affinity might be expected to have a similar geometry in the combining site and therefore have similar serological (or "idiotypic") properties. This argument

TABLE I. PARTIAL LIST OF IDIOTYPES SHARED BETWEEN T CELLS AND ANTIBODIES OF CORRESPONDING SPECIFICITIES

"Common name" of idiotypic marker	Specificity	Reported occurrence on	
		T cells	T-cell factors
NP	(4-Hydroxy-3-nitrophenyl)acetyl	Suppressor T-cell hybrid; specific T hybridomas (104)	Antigen-specific isolated receptor (4, 18, 19)
	Timothy allergen	Suppressors (20)	Antigen-specific (20) helper factor
HEL	Hen eggwhite lysozyme	Suppressors (21)	
(T,G)-A—L	Tyramyl-glutamyl-alanyl-lysine	Helper hybridomas (22)	Specific helper factor (23)
tyr(TMA)	L-Tyrosine-*p*-azophenyl-trimethylammonium	Antigen-binding T cells (24)	
ARS(Ar)	*p*-Azophenylarsonate	Antigen-binding T cells (25, 26)	Specific suppressor factors (27); biosynthetically labeled receptors (28, 29)
R 5,936	B6 anti-CBA antibodies (MHC specific)	Alloreactive T blasts (30)	Antigen-specific isolated receptor (30)
GAT	L-Glutamic acid ^{60}-L-alanine ^{30}-L-tyrosine^{10}		Specific suppressor factor (31, 32)
	Human γ globulin		Specific suppressor factor (33)
TEPC 15	Phosphoryl choline	Helpers (34, 35); delayed-type hypersensitivity (36)	
A5A	Streptococcal polysaccharide A	Helpers (4, 18) suppressors (4, 18)	
Nase	Staphylococcal nuclease	Helpers (37)	
	Directed against combining sites of rat anti-MHC alloantibodies	Alloreactive T cells (5)	Receptor (38, 39)
Idiotypes of human myeloma immunoglobulins	Unknown (40); anti-horse α_2-macroglobulin (41)	Peripheral T cells (40, 41)	Isolated idiotype-bearing receptors (40, 41)

is essentially one of convergence in which molecules lacking common ancestral genes might have evolved similar structures because of common function. The second explanation would be one of direct evolutionary homology. Since hundreds of millions of years of evolutionary time were occupied in the generation of a variable gene genetic system to generate antibody diversity, it is reasonable to expect that antigen-specific lymphocyte products including antibodies and T-cell receptors would express combining sites encoded by the variable region genes.

Possible examples indicating convergence have been reported, e.g., a sharing of idiotype between C-reactive protein and the phosphoryl choline-binding immunoglobulin HOPC 8 (44), the unexpected cross-reaction between antibodies to the TEPC 15 V_K light chain and the Thy-1 alloantigen (45), and the finding of a ubiquitous lymphocyte-associated protein which apparently shares the Ar cross-reactive idiotype as well as Ia antigenic determinants (46). However, *the more reasonable conclusion at this time is that the T-cell receptor molecules and factors express combining site determinants encoded by genes related to those of immunoglobulin heavy*

chain variable regions. This conclusion follows from the diversity of V_H-related idiotypes expressed by T cells. It appears possible that a sharing of "idiotype" might appear once or twice by chance; but it appears inherently improbable that such an event would happen at least 14 times. A second point which indicates that the combining sites of antigen-specific T-cell products most probably resemble immunoglobulin variable region-related idiotypes is that T cells recognize immunoglobulin idiotypes, whether expressed on cells or on antibody molecules (47, 50). Current data show that helper T cells and their products are specific for antigen and express the idiotype of the corresponding antibody (19, 23, 30), whereas suppressor T cells can be either specific for antigen and express idiotype (primary suppressors (27, 32)) or can react with idiotype, rather than with antigen (secondary suppressors (47–50)). Since idiotypes are usually formed as conformational determinants which require interaction between V_H and V_L (51–53), the fact that T cells and T-cell molecules (especially products of secondary suppressor T cells) can react with conformational determinants further suggests that the T-cell receptors, like antibody combining sites, recognize three-dimensional shapes. This issue is worth considering because it has been reported that T-cell antigen receptors differ from antibodies in recognizing short, linear stretches of amino acids in denatured proteins, as shown in immune response gene effects expressed in macrophages (54). The property of recognizing short linear stretches of amino acids resembles properties of proteases (55), rather than those of antibody.

The above data support a prima facie case for a sharing of idiotypic combining site determinants between antigen-specific T cells and their products, and antibodies of corresponding antigen specificities. In addition, although T-cell receptors and factors have not been found to express any of the known immunoglobulin constant regions, they have been shown to share a number of other properties with those of defined immunoglobulin variable regions. As of this time, there is persuasive evidence for a relationship between antigen-specific T-cell products and heavy chain variable regions, but the evidence for a relationship with light chain variable regions is much less certain. This particular problem is difficult to resolve because many of the idiotypic determinants studied above require interaction with the proper light chain variable region to form the combining site. Other properties shared between T-cell receptors and immunoglobulin heavy chain variable regions are as follows: association with allotype (30, 56), heterocliticity and fine structure for hapten binding (56), and nonidiotypic V_H-related determinants (57–60). Cramer *et al.* (56) maintain that the V_H regions of T-cell antigen-specific receptors share both framework and complementarity determining regions with V_H as expressed by B cells and antibodies.

T-cell hybridomas have been constructed which produce suppressor factors specific for the protein keyhole limpit hemocyanin and express a membrane-associated antigen-specific receptor. The specific suppressor factors bear an antigenic determinant detected using rabbit antisera made against the V_H region of the murine myeloma protein MOPC 315 (61). A point which will be considered below with respect to antigen-specific T-cell factors is the association of such functional molecules with products of the major histocompatibility complex; a finding which has been observed in the case of keyhole limpit hemocyanin-specific suppressor factors produced by hybridomas, and in the case of the (T, G)-A—L specific helper factor and the GAT-specific suppressor factor described in Table I above.

The present data indicate that *the spectrum of V_H-related molecules expressed by T cells is most probably not identical to that of the entire V_H pool, but may represent a restricted subset of this pool.* Evidence for this follows from the restricted expression of molecules related to the TEPC-15 idiotype by T cells (35), and from the expression of the NP idiotype by helper T cells and their factors. Apparently only V_H is required for the NP-idiotype of T cells,

whereas the serum antibody requires both the presence of the proper V_L and the proper V_H (56). In our hands, we find that some human T-cell tumor lines of amplifier phenotype express a V_H-related determinant which comprises about 5% of the total human V_H pool and most probably is defined by amino acid sequence lying between residues 23 and the end of the heavy chain variable region (J. J. Marchalonis, J. C. Hunt, G. R. Vasta, unpublished observations).

Table II presents a comparison between properties of T-cell variable regions and variable regions of antibodies or B cells. As described above, a number of idiotypic determinants shared with antibody molecules have been described for murine T cells and T-cell products, and idiotypic receptors have been described on human T cells. The rabbit differs from man and mouse in expressing allotypes in the variable region, and rabbit T-cell receptors have been described which bear the V_H allotype (62). It is worthwhile to note at this point that all investigators do not find V_H-related receptors on rabbit T cells. Jensenius *et al.* (63) do not find evidence for V_H (a allotype) markers occurring in the absence of light chains (b allotypes) by quantitative immunoassay, although they find large ($>10^4$ molecules/cell) numbers of Ig molecules in purified T-cell populations. They interpret their results to establish that V_H molecules found on T cells must represent B-cell contamination, and assert that all positive results obtained by other workers in any system must be due to either contamination of preparations with B cells or to use of poorly characterized antisera. Unfortunately, these workers present no data regarding the properties of an alternative recognition molecule. It is possible that the V_H determinant expressed on rabbit T cells and their products (62) is not a major a-allotype marker and that some antisera, thus, might not detect it. Furthermore, the rigid assumption that a-allotypes should be found on T cells only the complete absence of b-allotypes is unwarranted because the conformation of free V_H is different from that observed in the native state where it is noncovalently associated with V_L structures.

The murine T-cell receptor for the hapten NP resembles the NP-specific serum antibody of the same strain in showing a heteroclitic response in which the hapten nitroiodophenyl binds better than the original NP immunogen. Heterocliticity, like idiotype, is dependent upon the structure of the complementarity determining regions (hypervariable regions) within the antibody variable regions. In addition to idiotypic and heteroclitic properties, murine T cells and T-cell factors also express determinants which are associated with framework residues on murine heavy chain variable regions (56). Other determinants which can be localized to the heavy chain variable re-

TABLE II. COMPARISON BETWEEN PROPERTIES OF T-CELL AND ANTIBODY (B CELL) VARIABLE REGIONS

Feature of T-cell "variable" region	Association with antibody variable regions
(1) Idiotype (mouse, man)	V_H/V_L interaction (52); V_H (56); complementarity determining regions (56)
(2) Allotype (V_H)	Framework sequences (78) variable D-J-C_μ interaction (78); rabbit
(3) Heterocliticity ("fine structure"); mouse	Complementarity determining regions (56), e.g., anti-NP reacts better with NIP than with NP
(4) Framework, e.g., rabbit anti-mouse V_H	Framework sequences (79)
(5) Nonidiotype; restricted determinant	V_H nonidiotype, location unknown: mouse (79), man (58–60)
(6) Genetic linkage to C_H allotypes	Genetic linkage to C_H allotypes (5, 56, 80)

gion and which are not idiotypic have been described for T-cell products of mouse and man (57–59). Genes specifying idiotypic T-cell variable regions have been found to be genetically linked to allotypes of immunoglobulin constant regions (64–66) in a parallel fashion to that well established for V_H and C_H genes encoding immunoglobulins.

This section, which is based upon the serological properties of the antigen-specific T-cell receptor, indicates that T-cell antigen receptors possess a variable region exhibiting remarkable similarity to antibody variable regions. However, it is still possible that T-cell variable regions are *not* identical to the V_H structures expressed by antibodies. T-cell variable regions may represent a subset of the total V_H pool, or they may represent molecules similar to the primitive variable regions in evolution, rather than being directly homologous to antibody V_H structures in higher species. Moreover, although some T cells have been shown to produce messenger RNA for the constant region of μ chain, no evidence has yet been published describing V/D/J/CH rearrangements in T cells (67–69).

Receptors Versus Factors. *Although antigen specific soluble effector factors which mediate either helper or suppressor function might share the variable region combining site determinants with the antigen specific T-cell receptor and with the corresponding antibody, it does not necessarily follow that the constant regions or effector portions of the soluble molecules would be identical to those of the T cell surface antigen receptor.* By analogy with immunoglobulins, it would be expected that the antigen-specific molecules of T cells would represent distinct classes (isotypes) depending upon their function. Owen and her colleagues (64–66) have found evidence for allotypes which apparently are associated with constant regions of T cell derived molecules. These form a family of three genes, one encoding a suppressor factor, one specifying a helper T-cell product and the other encoding the surface receptor found on immature thymic T cells (64–66). Genetic studies have shown that these genes are linked to genes specifying murine immunoglobulin constant regions and that they map downstream from the locus specifying $C\alpha$ chains. A difference between receptors and factors has been defined by functional analyses: namely, *recognition defined as binding of antigen in solution is not dependent upon the MHC background of the T cells (70), whereas helper factors show a strong dependence upon the Ia (I-A) background (23) and some, but not all, antigen-specific suppressor factors require a functional association with products of I–J subregion (31, 71, 72).*

It is useful to consider the amounts of T-cell antigen-specific membrane receptors and soluble factors and to compare these quantities with other characterized membrane proteins found in lymphoid cells. Lymphocyte surface receptors usually comprise approximately 0.1 to 1% of membrane protein. This figure has been obtained for histocompatibility antigens (73), the Thy-1 alloantigen (74), and V_H-related receptors on primate T cells (75). The amount of a particular lymphocyte surface receptor computes to approximately 10,000 per cell. Factors can function at extremely small protein concentrations. For example, the monoclonal T-cell suppressor factor specific for GAT described by Krupen *et al.* (31) occurs at a calculated concentration of only 0.013 ng per mouse. This estimate represents the amount of active idiotype-bearing factor produced by a small fraction of the heterogeneous murine T-cell pool. The yield recovered from the monoclonal hybridoma line allows an estimation of the amount produced per cell. Krupen *et al.* (31) isolated two micrograms of GAT-specific suppressor factor from 6 liters of culture fluid. Assuming that the cells reached a reasonable density of 10^6/ml, and, using their molecular weight estimate of 24,000 for the specific factor, each cell on the average is calculated to release approximately 8000 molecules. This amount is consistent with those noted above for isolation of membrane receptors and is also congruent with previous observations that antigen-specific T-cell receptors are "shed"

or released from the cell surface via a metabolic process (76) which differs from the secretory process carried out by activated B cells or plasma cells. By contrast, a single plasma cell can secrete more than one million immunoglobulin molecules within an hour (77). *These calculations illustrate the difficulty inherent in obtaining large amounts of T-cell receptor because monoclonal T-cell lines express and release only the same amounts of receptor which are associated with normal T cells.* The hope of obtaining an immortalized T-cell line secreting large quantities (comparable to Ig secretion by plasma cells) has not yet been realized, although numerous antigen-specific monoclonal T-cell lines have been generated and studied (91).

Despite the relatively low yields of T-cell receptors and factors which can be isolated even from monoclonal T-cell lines, a remarkable amount of serological and molecular information has been generated for T-cell receptors and factors in recent years as summarized in Table III. This table presents data only on T-cell products which have been isolated by immune affinity chromatography and characterized to some degree by techniques such as polyacrylamide gel electrophoresis. The first five listings might be classified as T-cell surface-associated receptors which bear either idiotypic markers or nonidiotypic V_H determinants. In these five separate cases, no MHC-associated products have been detected on the isolated receptor, and a common theme is evident in that molecules of approximately the size of heavy chain (50–70,000 d) are consistently isolated. The remaining items in the table consist of factors which can be classified functionally as either antigen-specific helper or suppressor molecules which have been isolated either from sensitized normal T cells or from T-cell hybridomas. "IgT" helper factors have been described in the mouse (12) and the rat (13). The nature of the immunoglobulin-related determinant on these molecules is not clear, but these helper factors do not carry MHC determinants. Other helper T-cell factors have been shown to carry both I-A and V_H-related determinants (23), or unspecified immunoglobulin determinants such as those detected using chicken antibodies directed against the murine μ chain (81). Lonai *et al.* (82) have generated murine T-cell hybridomas producing helper factor directed against chicken gamma-globulin, and this factor expresses V_H framework determinants detected using rabbit antiserum against the V_H of MOPC 315, as well as I-A-associated determinants. A number of suppressor factors have recently been described; some of these consist of polypeptide chains of approximate mass 68,000–70,000 d and lack MHC components (84–89). On the other hand, the 68,000-d suppressor factor specific for keyhole limpit hemocyanin which is produced by a specific T-cell hybridoma described by Taniguchi *et al.* (61) apparently consists of two subunits. One of these has an approximate M_r of 45,000 d and bears V_H determinants whereas the other has an approximate mass of 25,000 and bears I-J markers. Evidence indicates that these subunits can be covalently linked by disulfide bonds in the "secreted" form, or non-covalently associated in factors extracted by cell lysis. The GAT-specific suppressor factor isolated by Krupen *et al.* (31) consists of a single chain of M_r 24,000 which is reported to carry both idiotypic and I-J markers. Although there has been considerable interest in the production and specificity of factors directed against the arsonate hapten because of the existence in A/J mice of a cross-reactive idiotype, the status of factors in this system is not clear. Both normal sensitized T cells (25, 26) and monoclonal T-cell hybridomas bearing the cross-reactive idiotype have been described (29), but confusion has resulted in regard to the nature of factors produced. One group reports the isolation, by affinity chromatography on the arsonate hapten, of a single chain molecule of mass 92,000 d which does not express V_H or MHC determinants (90), and another group reports the isolation of the single chain of approximate mass 62,000 daltons which expresses both idiotype and I-J determinants (defined using alloantisera; 29). However, the latter molecule is found in all lymphocytes (46). In our investiga-

TABLE III. PROPERTIES OF ISOLATED T CELL RECEPTORS AND FACTORS

Source	Specificity	V_H determinants	MHC	Function	Organization
Rat alloreactive T cells (5)	Alloantigens	Idiotype	NIL	Receptor	Polypeptide of M_r 70,000; can form disulfide-bonded dimers (38, 39)
Mouse alloreactive T cell (30) Lyt-1$^+$ 2,3-	Alloantigens	Idiotype (5936) allotypes (Ig-1^b)	NIL	Receptor	Single, nonglycosylated polypeptide chain; M_rs observed: 50,000; 62,000 and 75,000; "domains"
Marmoset T-cell line/ amplifier phenotype (58)	Unknown	Restricted V_H determinant shared with human myeloma heavy chains	NIL	Receptor (?)	Polypeptide of M_r 68,000–70,000; can form disulfide-bonded dimers
Human peripheral T cells	Unknown	Idiotype (40, 41); nonidiotypic V_H determinant (58–60)	NIL	Receptor (?)	Polypeptide of M_r 70,000
Sensitized mouse helper T cells (19, 56)	NP	Idiotype (V_H associated)	NIL	Receptor	M_r 150,000 (gel filtration; heavy chains (M_r 50,000), light chains (M_r 25,000)
Murine helper T cells (12)	Keyhole limpit hemocyanin (KLH)	Unspecified Ig determinants probably V_H framework (17)	NIL	Helper	M_r approx. 180,000; disulfide-bonded heavy chains (M_r 70,000)/light chains (M_r 25,000), disulfide linked (?)
Murine antigen-specific T-cell hybridoma (61)	KLH	V_H framework	I-J	Suppressor factor	M_r 68,000; I-J bearing chain (M_r 25,000) disulfide ("secreted") or non-covalently ("extracted") linked to V_H-bearing chain (M_r 45,000)
GAT-specific murine T-cell hybridomas (31)	GAT	GAT idiotype	I-J	Suppressor	Single chain M_r 24,000 carrying Id and I-J markers
CG-specific murine T-cell hybridomas (82, 83)	Chicken γ globulin	V_H framework	I-A	Helper	Major component M_r 60–70,000; minor components M_rs 35,000, 30,000; higher components (140,000; 300,000) (83)
Murine suppressor T cells (84–86)	DNP or TNP	Not tested	NIL	Suppressor delayed-type hypersensitivity	Single chain M_r 68,000
Murine suppressor T cells (87–89)	Sheep erythrocyte glycophorin	F_V	NIL	Suppressor	Single chain M_r 68,000
Murine T-cell hybridoma of suppressor phenotype (29)	ARS	Id	I-J	?	Single chain M_r 62,000; ubiquitous molecule (29)
	ARS	NIL	NIL	?	Single chain, M_r 92,000 (90)
Stimulated murine peripheral T cells (26)	ARS	Id	?	?	Single chain M_r 68,000 (28); also reacts with chicken antibody to murine Fab fragment

tions of an idiotype-bearing molecule biosynthetically produced by stimulated murine peripheral T cells, we have isolated, under reducing conditions, a single chain of 68,000 d which also reacts with chicken antibody directed against the Fab fragment of murine immunoglobulin (26, 28). At this point in time, it can only be said that the results in the arsonate system are inconclusive and further analysis of antigen binding molecules is required, particularly because of the report that a widely distributed molecule of unknown function apparently binds arsonate and expresses a cross-reactive idiotype (46).

Evidence obtained from murine (71) and primate systems (58, 59) indicates that a subpopulation of normal peripheral T cells (approximately 30% of PTL) expresses V_H-related determinants (58, 59), and that this subpopulation also tends to express I region markers (71, 92). The studies summarized above indicate that the capacity of a T cell or a released molecule to combine with antigen results from the presence of a V_H-related component, not from the presence of an MHC marker. Because we have at hand human and lower primate T cell lines which express V_H-related surface components as well as HLA-Dr (the human equivalent of Ia markers), we carried out studies designed to determine whether or not the I region and V_H-bearing components existed as a functional unit on the cell surface. Investigations involving direct isolation of either V_H-bearing (58) or HLA-Dr-bearing components (92) indicated that anti-V_H antibodies isolated components of approximately 70,000 daltons, whereas monoclonal anti-Ia isolated components of 28,000 and 32,000 d. We did not find evidence for a covalent or a strong noncovalent association between these two components in the form in which they are released into the culture fluid or as they are expressed on membrane fragments. In codistribution analyses using double immunofluorescence, we found a lack of congruity between HLA-Dr and V_H products (D. DeLuca and J. J. Marchalonis, unpublished observations). Although we cannot exclude the transient association of components of MHC and V_H systems, we did not find any evidence indicating a strong linkage between the two sets of surface components.

At this point in time, it is reasonable to conclude that the antigen-specific recognition moiety on the T-cell receptor and released effector factors is a V_H-related marker. A good deal of evidence now suggests that this component most probably has an intact subunit mass of 68–70,000 d. This component binds antigen, and bears idiotype. Evidence also indicates that it (by itself) can bind to the surface of macrophages (1), presumably by some sort of constant region structure which can bind to a macrophage receptor (which is analogous to an Fc receptor). The association of V_H-bearing products with MHC products in the generation of helper or suppressor factors appears to be involved in situations which require cell/cell interaction, and might also be expected to depend upon the type of suppressor factor under consideration. For example, helper and suppressor factors would be expected to have different constant regions adapted for their particular effector functions, which would imply the existence of different MHC associations and different types of cell/cell interactions. Furthermore, it might be expected that primary suppressor factors (which bear idiotype and are antigen specific) could differ from secondary suppressors (which are anti-idiotypic) which differ in their combining site specificity also could differ in MHC restrictions. This situation is analogous to that of antibody heavy chains where structurally and functionally distinct heavy chains (e.g., γ chain and ϵ chain) can share the same V_H structure.

Tentative Model for Receptor and Factor Structures. A number of significant issues remain to be resolved regarding the nature of T-cell receptors and factors. Among the most prominent of these are as follows: *The valence or number of combining sites of intact T-cell antigen receptor in the absence of denaturing solvents is unresolved.* Since the isolated T-cell factors will neutralize hapten-derivatized bacteriophage, the number of combining sites on the molecule or molecular complex must

be at least two (93), and the size of these isolated receptors as estimated by gel filtration is approximately 150,000, a value which would correspond to a dimer of heavy chains. *The requirement for light chain variable regions with the T-cell receptor is unclear.* Many of the antisera directed against idiotypes or V_H region determinants which react with T-cell receptor structures have a strong dependence upon the association of V_H and V_L for detection (51–53). In some cases, polypeptide chains resembling light chains have been isolated and partially characterized (1, 6, 14), although these molecules have been shown to be serologically distinct from standard κ or λ chains (17, 94) and they usually express a nominal molecular weight slightly higher than that usually observed for light chains. Third, the *detailed molecular properties of T-cell variable regions and their similarity to immunoglobulin variable regions both at the polypeptide and nucleic acid levels remains to be established.* This is a fundamental problem, and we will consider it in detail below. Even though antigen-specific T-cell products express a variable region serologically related to Ig V_H, the exact degree of homology can be answered only by detailed sequence analysis of the polypeptide and its gene.

Despite these questions to be resolved, a consensus regarding the properties of the V_H-related T-cell receptor is emerging from characterization studies being performed in many laboratories which were cited in Table III above. In particular, there is general agreement that T-cell receptors (and factors) bear serologically detectable variable regions and constant regions. Constant regions apparently unique to T-cell products have now been detected using alloantisera (64–66, 95, 96), xenoantisera (97), and hybridoma antibodies (98) produced against isolated T-cell products of man and rodent species. The T-cell constant regions are distinct from the immunoglobulin isotypic determinants, but evidence now exists for the presence of a family of related isotypes of T-cell products (64–66, 95). These will be considered in detail below. It has recently become possible to isolate (by immune affinity chromatography) sufficient quantities of T-cell products (approximately 100 μg) to allow initial molecular characterization studies. Table IV presents a comparison of the amino acid compositions of V_H-related T-cell products isolated from a monoclonal T-cell hybridoma producing GAT-specific suppressor factor (31), a long-term *in vitro* grown marmoset T-cell line of amplifier phenotype (75, 99), and an idiotype bearing murine T-cell product (30). The molecules show an overall similarity, particularly in acidic amino acids, basic amino acids, and in the hydrophobic amino acids isoleucine, leucine, tyrosine, and phenylalanine. They are very similar in the hydroxylic amino acid threonine.

Sufficient quantities of the V_H-bearing T-cell products (receptor and certain factors) have been isolated to allow characterization studies using standard techniques of protein chemistry in order to compare the structures of the T-cell molecules with one another and with classical immunoglobulin chains. A number of serologically and functionally characterized fragments have recently been generated

TABLE IV. COMPARISON OF AMINO ACID COMPOSITIONS OF V_H-RELATED T-CELL PRODUCTS

	Residues/100 residues		
Amino acid	70-N2(τ)[a]	GAT-TsF[b]	Tcr[c]
Asx	12.7	9.0	10.1
Thr	5.1	5.1	5.4
Ser	10.5	7.9	6.7
Glx	14.8	18.3	12.8
Pro	6.7	N.D.	5.0
Gly	N.D.	12.8	6.5
Ala	10.5	7.1	8.1
Val	6.2	5.5	6.7
Met	0.7	1.0	2.5
Ile	3.1	2.5	3.0
Leu	7.5	7.0	9.5
Tyr	2.6	4.0	3.0
Phe	3.4	3.3	4.2
His	3.2	2.5	3.2
Lys	8.5	7.4	7.4
Arg	4.7	6.6	3.6

[a] Data of J. J. Marchalonis, J. C. Maxwell, and C. Schwabe (unpublished observations) for V_H-related product of the *in vitro* marmoset amplifier T-cell 70-N2.

[b] Data of Krupen *et al.* (31) for GAT-specific suppressor factor from murine T-cell hybridoma.

[c] Data of Rubin *et al.* (30) for the idiotype-bearing allospecific murine T-cell receptor.

(30, 39, 75, 86, 89) by cleavage with specific proteolytic enzymes or with CNBr. Figure 1 presents data from this laboratory showing the intact 68,000-d product of a human T-cell line YT4E (Fig. 1A, lane 1) and the cleavage products generated by tryptic proteolysis of this T-cell product (Fig. 1B, lane 1) and of the corresponding product of the amplifier T-cell line 70-N2 (Fig. 1B, lane 2). Major fragments in the range 20–25,000 d are generated as is a major fragment of approximate mass 45–47,000 d. In addition, a number of higher-molecular-weight fragments are observed. The fragments in the molecular weight range 20–25,000 d react with antisera directed against V_H determinants; the major fragment of approximate mass 45,000 d is not precipitated by

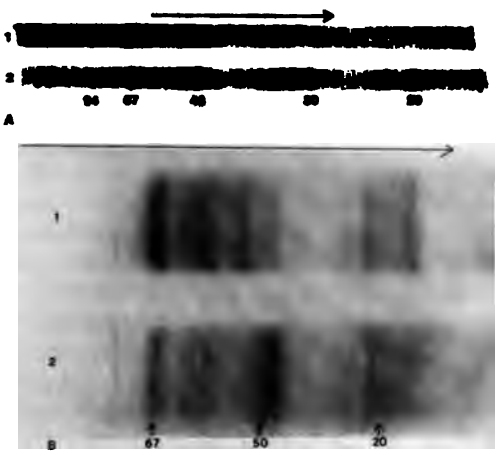

FIG. 1. Analysis by polyacrylamide gel electrophoresis under reducing conditions of intact (A) V_H-associated T-cell product and fragments produced by tryptic proteolysis of the molecule (B). (A) Lane 1: the 68,000-d component produced by the human *in vitro* grown T-cell leukemia line YT4E. This component was isolated from formic acid-solubilized membrane preparations by immune affinity chromatography using goat antisera directed against the Fab monomer fragment of a human IgM myeloma protein. Lane 2: molecular weight standards having masses as indicated on the gel. (B) Lane 1: tryptic fragments of the 68,000-d V_H-related product of the human T-cell lymphoma line YT4E. Lane 2: tryptic fragments of the 68,000-d V_H-related molecule produced by the marmoset *in vitro* T leukemia line of amplifier phenotype 70-N2. V_H-related products were isolated and digested as described in ref. (75).

anti-V_H reagents. These V_H-related products of two separate T-cell lines are serologically related, but are not identical to one another. In addition (Fig. 1B), their tryptic fragments give similar but not identical patterns. Our data and those recently generated by other laboratories (30, 39, 86, 89) are summarized in Fig. 2 which gives a schematic diagram illustrating observed fragments of V_H bearing T-cell receptors. The τ chain undergoes a fragmentation pattern which indicates the presence of domains of approximate mass 12,000 d (30, 75). A V_H-bearing fragment (of approximately 24,000 d) comparable to an Fd fragment of heavy chain has been observed (75), as has a fragment of about 45–47,000 d (75, 89) which expresses effector functions similar to that shown by the Fc fragment of heavy chains (89). Notably, the Fc-like fragment of suppressor factors apparently expresses nonspecific suppressor activities (89). In addition, by following cleavage using CNBr with immune affinity chromatography it has been possible to isolate an antigenic fragment of approximate mass 12,000 d which most probably corresponds to the T-cell heavy (τ) chain variable region (75). In the cases which have been studied, the isolated τ chains have had blocked N-terminuses (1, 30, 75), a result which might indicate some relationship to either the V_HI or V_HII subgroups of heavy chains. Rubin reports that his murine idiotype bearing T-cell receptor lacks detectable carbohydrate (30), but this question has to be resolved for other T-cell receptors and factors. Using papain digestion, we (J. C. Hunt, J. J. Marchalonis, unpublished observations) have recently isolated a fragment of approximate mass 7800 d which is hydrophobic, as assessed by elution behavior from reverse phase columns (by high-performance liquid chromatography) and amino acid composition analysis. This peptide reacts with antisera directed against Fab region and V_H region determinants. It is possible that this fragment represents the disulfide-bonded loop of the variable region, and studies are in progress to establish its identity. The structure illustrated here is based upon

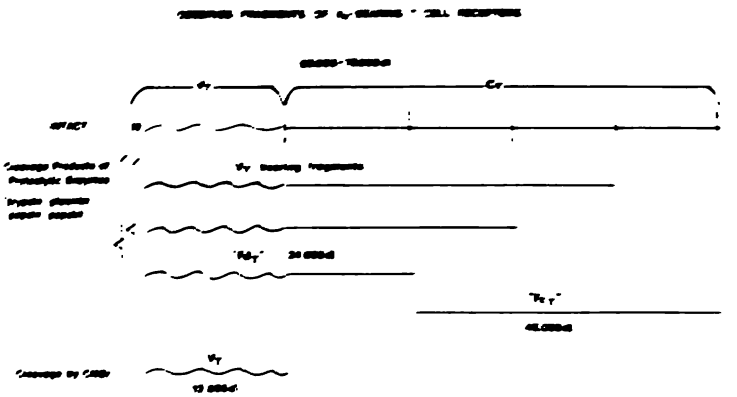

FIG. 2. Schematic diagram illustrating the proposed domain structure of V_H-bearing T-cell receptors and the organization of observed antigenic and functional fragments produced by proteolysis or chemical cleavage. This diagram pertains only to the V_H-bearing, non-MHC chain. It is likely that I-region products can be associated as separate chains with molecules of this nature in the formation of active factors or a possible recognition/activation complex on the cell surface. Documentation regarding these fragments is given in the text.

studies of receptors and the suppressor factors which are of approximate mass 68–70,000 d and lack strongly associated MHC products. It is possible that either I-A or I-J region products are associated with functional T-cell factors either as a second polypeptide associated through noncovalent linkage or through disulfide bonds. It is frequently found that products of separate chromosomes can form noncovalent multimers (hemoglobins) or disulfide-bonded assemblies (immunoglobulins). However, the question of a single polypeptide (31) expressing both MHC-associated regions (chromosome 6 in man) and immunoglobulin-associated regions (V_H; chromosome 14 in man) is a most interesting and unique one and requires further detailed analysis.

Possible Relationships between T-Cell Receptor and Immunoglobulin Genes. *Although the serological data marshalled above on idiotypes and other V_H markers indicates that antigen-specific T-cell receptors possess a combining site region showing remarkable similarity to immunoglobulin variable regions (Tables I and II above), a paradox remains because no definitive reports have yet appeared which establish that T cells possess genes consisting of rearranged V_H, D, J, and immunoglobulin CH regions (67–69).* It is now generally accepted that T cells do not express standard immunoglobulin heavy chain constant region isotypes, but it might intuitively be expected that the heavy chain expressed by T cells would show the same sort of V_H, D, J, C rearrangement that immunoglobulins do. This arrangement has not been demonstrated, and it may be that either the V_H-like products of T cells are not encoded by immunoglobulin V_H genes, or that a different arrangement or type of rearrangement has occurred in the generation of T-cell products. One important result of the serological studies which should be stressed here is that there are indications that the repertoire of V_H structures expressed by T cells most probably represents a subset of the total V_H population (35, 36), J, J. Marchalonis, J. C. Hunt, G. R. Vasta, A. C. Wang, unpublished observations). An important series of recent experiments directly germane to this question revealed constant region allotypes on products of T suppressor cells (64, 96), T helper cells (65, 96), and on T-cell primitive receptors (66). Owen and her colleagues have described a series of alleles for T-cell markers which map downstream from the C_α locus of the immunoglobulin chain cluster. Furthermore, Tokuhisa and Taniguchi (96) have also recently described two distinct allotypic determinants on the antigen-

specific suppressor and enhancing T-cell factors that are encoded by genes linked to the immunoglobulin heavy chain locus. Figure 3 presents a hypothetical model that delineates alternatives which might account for the observed observations. It has been proposed that the constant region of the T-cell receptor heavy chain, the τ chain, might be very similar to the primitive heavy chain in immunoglobulin evolution (1, 100) and therefore the C_τ gene might be located between the V_H cluster and the group of D genes. This location (position I), with either a different set of D-like or J-like genes or a lack of them, would account for present observations that V, D, J, and C rearrangements have not been found in T cells. Another major site on the immunoglobulin heavy chain chromosome for the location of T-cell receptor genes is shown in position II which follows from the studies of Owen and her collaborators (64–66). The constant regions of the suppressor, amplifier, and thymocyte antigen receptor are located to the right of the standard immunoglobulin isotype genes. This arrangement has been questioned because, if standard V, D, J, and C rearrangement occurred within the T cells, it would be predicted that the entire collection of immunoglobulin constant region genes would be deleted in T-cell maturation. This is not the case; in fact, T cells have $C\mu$ genes (101). It is possible, however, that a subset of V_H genes was duplicated and translocated to a position between the standard C_H genes and the C_τ cluster. Because of the enormous interest and the vigor of the attack on the genetic location of T-cell receptor genes, it is anticipated that the exact solution to this problem will be found in the near future.

Conclusions. The problem of the molecular nature of the T-cell receptor for antigen is one of the major unresolved issues in contemporary immunology. A consensus is developing regarding the existence of recognition structures related to immunoglobulin heavy chain variable regions on the surface of certain functional T cells and on many of the properties of isolated receptor (and factor) structures. A molecule of approximate subunit mass 68,000 d has been isolated from certain primate and rodent T cells and has been subjected to controlled proteolysis using various proteolytic enzymes and standard chemical cleavage methods. This molecule can be degraded into a major fragment of M_r 24,000, which bears the V_H determinant and binds antigen, and to a fragment of approximate mass 45–47,000 d which lacks V_H determinants

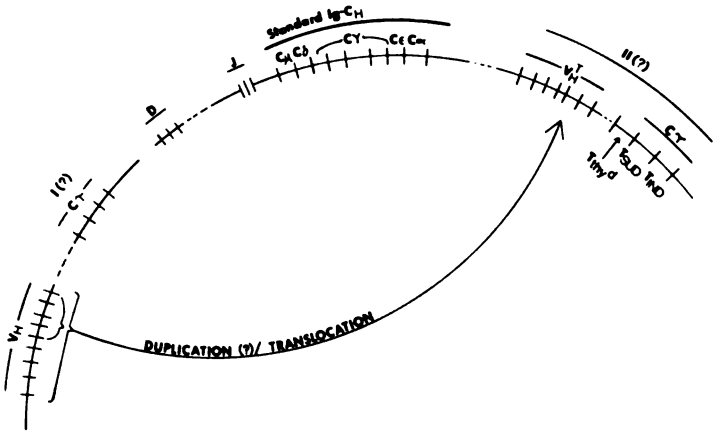

FIG. 3. Hypothetical model depicting possible arrangements of T-cell receptor variable and constant region genes within the immunoglobulin heavy chain gene cluster. The constant region of the T-cell heavy chain is designated C_τ. The markers $T_{thy}{}^d$, T_{sud}, and T_{ind} are the T-cell allotypic constant region markers defined by Owen and her colleagues (64–66). I (?) and II (?) indicate possible positions at which the C_τ genes can be located.

and carries out nonspecific effector functions. In addition, larger V_H-bearing fragments as well as subfragments of the V_H have been isolated and partially characterized. Although this molecule does not share constant region determinants with immunoglobulin heavy chains, the present data suggest that the molecule resembles heavy chains in being formed of domains of approximate mass 12,000 d. Genetic mapping studies indicate that the V_H structure associated with T-cell receptors maps with immunoglobulin V_H, although the genes encoding T-cell V_H structures most probably represent a subset of the total V_H pool available to immunoglobulin heavy chains. It is also possible that the T-cell variable regions and constant regions represent direct lineal descendants of primitive immunoglobulins in evolution and, therefore, would show a closer sequence homology to immunoglobulins of lower vertebrates rather than to those of man and rodents. Further amino acid sequence data and nucleic acid sequence data are required to test this hypothesis. The T-cell receptor molecule apparently possesses a blocked N-terminus. Although amino acid compositions have now been obtained for three V_H-bearing T-cell molecules, amino acid sequence data are lacking. Major issues which remain to be resolved are the direct demonstration using nucleic acid probes that the T-cell V_H genes lie within the immunoglobulin V_H cluster and the location of the constant region genes of the antigen-specific T-cell receptor. The genes that specify the factors likewise remain to be precisely mapped, although it is likely that antigen-specific receptors and factors share the same V_H pool.

One of the major features which is often taken to distinguish "recognition of antigen" by T cells from that of antibodies and B cells is that the T-cell recognition process is usually considered to show restrictions imposed by the major histocompatibility complex whereas antibodies show no such constraints. Based upon the MHC restriction in T-cell recognition, it has been proposed that T cells either express one surface receptor capable of recognizing both MHC products and nominal antigens (single receptor), or two distinct receptors (dual recognition hypothesis), one recognizing antigen and the other recognizing MHC products. Antigen-specific T-cell hybridomas expressing two specificities for nominal antigen and two MHC backgrounds have been constructed in the attempt to determine whether or not V_H and MHC products assort independently in T-cell function (102, 103). The present results are inconclusive, however, because one group finds conjunct association (102), but the other provides evidence of independent functional distribution suggesting dual recognition (103). The molecular data reviewed here suggest that the V_H-bearing/non-MHC-associated structure most probably binds antigen on the cell surface and is the antigen recognition unit in T-cell factors, whereas an association between this molecule and I-A- or I-J-specified products might be required for the production of active factors involved in cell/cell interactions. Substantial progress has been made in the molecular characterization of the elusive T-cell antigen receptor, although a formidable task remains in determining the exact mechanisms of antigen-driven activation of T cells and cell/cell collaboration.

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1. Cone RE. Molecular basis for T lymphocyte recognition of antigen. Prog Allergy 29:182–221, 1981.
2. Marchalonis JJ. The T cell antigen receptor: The minimal hypothesis revisited. Immunol Today 3:10–17, 1982.
3. Tada T, Okumura D. The role of antigen-specific T cell factors in the immune response. Advan Immunol 28:1–87, 1979.
4. Rajewsky K, Eichmann K. Antigen receptors of T helper cells. Contemp Topics Immunobiol 7:69–12, 1977.
5. Binz H, Wigzell H. Antigen-binding, idiotypic T-lymphocyte receptors. Contemp Topics Immunobiol 7:113–177, 1975.
6. Marchalonis JJ, Cone RE. Biochemical and

biological characteristics of lymphocyte surface immunoglobulin. Transplant Rev 14:3–49, 1973.
7. Moroz C, Lahat N. Surface immunoglobulin of mouse thymus cells and its *in vitro* biosynthesis. Cell Immunol 13:397–406, 1974.
8. Boylston AW, Watson SR, Anderson RL. Mouse T cell tumor immunoglobulin. I. Antigenic properties and effects on T cell responses. Immunology 31:827–835, 1976.
9. Putnam D, Clagett J, Storb U. Immunoglobulin synthesis by T cells. Quantitative aspects. J Immunol 124:902–912, 1980.
10. Singh G, Bazin H, Ladoulis C. *In vitro* synthesis and release of immunoglobulins by thymocytes of inbred rats. Mol Immunol 16:755–766, 1979.
11. Hammerling U, Pickel HG, Mack C, Masters D. Immunochemical study of an immunoglobulin-like molecule of murine T lymphocytes. Immunochemistry 13:533–538, 1976.
12. Feldmann M, Cone RE, Marchalonis JJ. Cell interactions in the immune response *in vitro*. VI. Mediation by T cell surface monomeric IgM. Cell Immunol 9:1–11, 1973.
13. Taniguchi M, Tada T. Regulation of homocytotropic antibody formation in the rat. X. IgT-like molecule for the induction of homocytotropic antibody response. J Immunol 113:1757–1769, 1974.
14. Moseley JM, Marchalonis JJ, Harris AW, Pye J. Molecular properties of T lymphoma immunoglobulin. I. Serological and general physical chemical properties. J Immunogene 4:233–248, 1977.
15. Marchalonis JJ, Warr GW, Santucci LA, Szenberg A, von Fellenberg R, Burckhardt JJ. The immunoglobulin-like T cell receptor IV. Quantitative cellular assay and partial characterization of a heavy chain cross reactive with the Fd fragment of serum mu chain. Mol Immunol 17:985–999, 1980.
16. Warr GW, Marton G, Szenberg A, Marchalonis JJ. Reactions of chicken antibodies with immunoglobulins of mouse serum and T cells. Immunochemistry 15:615–622, 1978.
17. Cone RE, Rosenstein RW. Isolation of T cell membrane proteins (IgT) with antisera to non-isotypic determinants of immunoglobulins: Evidence that IgT "light" chains are not identical to B cell kappa chains. Mol Immunol 18:67–77, 1981.
18. Eichman K. Expression and function of idiotypes on lymphocytes. Advan Immunol 26: 195–254, 1978.
19. Cramer M. T cell factors-T cell receptors. In: Steinberg CM, Lefkovitz I, eds. The Immune System: Basel, Karger, Vol. I: pp311–315, 1981.
20. Malley A, Brandt CJ, Deppe LB. Preparation and characterization of the antiidiotypic properties of rabbit anti-timothy antigen B helper factor and antimouse timothy IgE antisera. Immunology 45:217–225, 1982.
21. Harvey MA, Adorini L, Miller A, Sercarz EE. Lysozyme-induced T-suppressor cells and antibodies have a predominant idiotype. Nature (London) 281:594–596, 1979.
22. Eshhar Z, Apte RN, Lowry I, Ben-Neriah Y, Givol D, Mozes E. T cell hybridoma bearing heavy chain variable region determinants producing (T, G)-A—L-specific helper factor. Nature (London) 286:270–272, 1980.
23. Mozes E, Haimovich J. Antigen specific T cell helper factor cross reacts idiotypically with antibodies of the same specificity. Nature (London) 278:56–57, 1979.
24. Prange CA, Fiedler J, Nitecki DE, Bellone CJ. Inhibition of T-antigen-binding cells by idiotypic antisera. J Exp Med 146:766–778, 1977.
25. Lewis GK, Goodman JW. Purification of functional determinant-specific idiotype-bearing murine T cells. J Exp Med 148:915–924, 1978.
26. Warr GW, DeLuca D, Marchalonis JJ. The immunoglobulin-like T cell receptor. III. Binding of the arsonate hapten by idiotype-bearing T cells and antibody is blocked by avian antibody to $(Fab')_2$. Mol Immunol 16:735–738, 1979.
27. Nisonoff A, Greene MI. Regulation through idiotypic determinants of the immune response to the p-azophenylarsonate hapten in strain A mice. In: Fougereau M, Dausset J, eds. Progress in Immunology IV. New York, Academic Press, pp58–80, 1980.
28. Marchalonis JJ, Warr GW, Hunt JC, Wang AC, Decker JM, DeLuca D. Murine and primate T cell-derived proteins related to immunoglobulin combining site-region determinants. In: Janeway C, Sercarz EE, Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp501–507, 1981.
29. Pacifico A, Capra JD. T cell hybrids with arsonate specificity. I. Initial characterization of antigen specific T cell products that bear a cross-reactive idiotype and determinants encoded by the murine major histocompatibility complex. J Exp Med 152:1289–1301, 1980.
30. Rubin B, Suzan M, Kahn-Perles B, Bayor C, Schiff C, Bourgois A. Isolation and biochemical characterization of idiotype-bearing T-cell receptors which express private allotypes and/or isotypes: A review. Bull Inst Pasteur 78:305–346, 1980.
31. Krupen K, Araneo BA, Brink L, Kapp JA, Stern S, Weider KS, Webb DR. Purification and characterization of a monoclonal T-cell suppres-

sor factor specific for poly (LGlu^{60} LAla^{30}LTyr^{10}). Proc Nat Acad Sci USA 79:1254–1258, 1982.

32. Germain RN, Ju ST, Kipps TJ, Benacerraf B, Dorf ME. Shared idiotypic determinants on antibodies and T-cell derived suppressor factor specific for the random terpolymer L-Glutamic acid ^{60}-L-Alanine^{30}-L-Tyrosine^{10}. J Exp Med 149:613–622, 1979.
33. Chaouat G. Human gammaglobulin tolerance: Shared idiotypy of T-cell suppressor factor and antibodies. Ann Immunol (Inst Pasteur) 131:91–95, 1980.
34. Julius MH, Cosenza H, Augustin AA. Evidence for the endogenous production of T cell receptors bearing idiotypic determinants. Eur J Immunol 8:484–491, 1978.
35. Benca R, Quintans J, Kearney JF, Flood PM, Schreiber H. Studies on phosphorylcholine-specific T cell isotopes and idiotype-specific immunity. Mol Immunol 17:823–831, 1980.
36. Sugimura K, Kishimoto T, Maeda K, Yamamura Y. Demonstration of T15 idiotype-positive effector and suppressor T cells for phosphorylcholine-specific delayed-type hypersensitivity response in CBA/N or (CBA/N X Balb/C)F_1 male mice. Eur J Immunol 11:455–461, 1981.
37. Nadler PI, Miller GG, Sachs DH, Hodes RJ. The expression and functional involvement of nuclease-specific idiotype on nuclease-primed helper T cells. Eur J Immunol 12:113–120, 1982.
38. Binz H, Wigzell H. Shared idiotypic determinants on B and T lymphocytes reactive against the same antigenic determinants.V. Biochemical and serological characteristics of naturally occurring, soluble antigen-binding T-lymphocyte-derived molecules. Scand J Immunol 5:559–571, 1976.
39. Binz H, Wigzell H. T cell receptors with allo-major histocompatibility complex specificity from rat and mouse. Similarity of size, plasmin susceptibility and localization of antigen-binding region. J Exp Med 154:1261–1278, 1981.
40. Lea T, Forre Ø, Michaelsen TE, Natvig JB. Shared idiotypes on human peripheral blood B and T lymphocytes. J Immunol 122:2413–2417, 1979.
41. Preud'Homme JL, Klein M, Labourne S, Seligmann M. Idiotype-bearing and antigen-binding receptors produced by blood T lymphocytes in a case of human myeloma. Eur J Immunol 7:840–846, 1977.
42. Kishimoto T, Suemura M, Sugimura K, Okada M, Nakanishi K, Yamamura Y. Characterizations of T cell-derived immunoregulatory molecules from murine or human T hybridomas. In: Feldmann M, Schrier MH, eds. Lymphokines, Vol 5, Monoclonal T Cells and Their Products. New York, Academic Press, pp129–160, 1982.
43. Kapp JA, Araneo BA, Clevinger BL. Suppression of antibody and T cell proliferative responses to L-glutamic acid 60-L-alanine 30-L-tyrosine 10 (GAT) by a specific monoclonal T cell factor. J Exp Med 152:235–240, 1980.
44. Volanakis JE, Kearney JF. Cross-reactivity between C-reactive protein and idiotypic determinants on a phosphocholine-binding murine myeloma protein. J Exp Med 153:1604–1614, 1981.
45. Pillemer E, Weissman IL. A monoclonal antibody that detects a V_K-TEPC15 idiotypic determinant cross-reactive with a Thy-1 determinant. J Exp Med 153:1068–1079, 1981.
46. Clark AF, Capra JD. Ubiquitous nonimmunoglobulin p-azobenzenearsonate-binding molecules from lymphoid cells. J Exp Med 155:611–616, 1982.
47. Sherr DH, Ju ST, Dorf ME. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XII. Fine specificity of anti-idiotypic suppressor T cells (TS_2). J Exp Med 154:1382–1389, 1981.
48. Minami M, Okuda K, Furusawa S, Benacerraf B, Dorf ME. Analysis of T cell hybridomas. I. Characterization of H-2 and Igh-restricted monoclonal suppressor factors. J Exp Med 154:1390–1402, 1981.
49. Sy MS, Nisonoff A, Germain RN, Benacerraf B, Greene MI. Antigen- and receptor-driven regulatory mechanisms VIII. Suppression of idiotype-negative, p-azobenzenearsonate-specific T cells results from the interaction of an idiotype-specific second order T suppressor cell with a cross-reactive-idiotype-positive, p-azobenzenearsonate-primed T cell target. J Exp Med 153: 1415–1525, 1981.
50. Bona C, Paul WE. Cellular basis of regulation of the expression of idiotype. I. T cell suppressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. J Exp Med 149:592–600, 1979.
51. Sher A, Lord E, Cohn M. Reconstitution from subunits of the hapten binding sites and idiotypic determinants of mouse anti-phosphorylcholine myeloma proteins. J Immunol 107:1226–1234, 1971.
52. Schiff C, Boyer C, Milili M, Fougereau M. The idiotypy of the MOPC 173 (IgG_{2b}) mouse myeloma protein: Characterization of syngeneic, allogeneic and xenogeneic anti-idiotypic antibodies. Contribution of the H and L chains to the idiotypic determinants. Eur J Immunol 9:831–841, 1979.
53. Marchalonis JJ, Warr GW, Smith P, Begg GS,

Morgan FJ. Structural and antigenic studies of an idiotype-bearing murine antibody to the arsonate hapten. Biochemistry 18:560–565, 1979.

54. Rosenthal AS. Determinant selection and macrophage function. Immunol Today 3:33–34, 1982.
55. Barrett, AJ, McDonald, JK. Mammalian proteases: A glossary and bibliography. Vol I, Endopeptidases. New York, Academic Press, 1980.
56. Cramer M, Reth M, Grutzmann R. T cell V_H versus B cell V_H. In: Janeway C, Sercarz EE, Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp429–439, 1981.
57. Ben Neriah Y, Givol D, Lonai P, Simon MM, Eichmann K. Allotype-linked genetic control of a polymorphic V_H framework determinant on mouse T-helper cell receptors. Nature (London) 285:257–259, 1980.
58. Marchalonis JJ, Warr GW, Rodwell JD, Karush F. Surface component of primate thymus-derived lymphocytes related to a heavy chain variable region. Proc Nat Acad Sci USA 77:3625–3629, 1980.
59. Førre Ø, Michaelsen T, Natvig J. Nonidiotypic V_H antigens can be detected on human lymphocytes. Clin Immunol Immunopathol 22:436–441, 1982.
60. Michaelsen TE, Lea T. Sheep, rabbit and chicken antisera against a human V_H fragment: Reactivity with immunoglobulins and lymphocytes. Immunology 45:751–759, 1982.
61. Taniguchi M, Saito T, Takei I, Kanno M, Tokuhisa T, Tomioka H. Suppressor T cell hybridomas and their soluble products. In: Feldmann M, Schrier MH. Lymphokines, Vol. 5, Monoclonal T Cells and Their Products. New York, Academic Press, pp77–117, 1982.
62. Krawinkel U, Cramer M, Mage RG, Kelus AS, Rajewsky K. Isolated hapten-binding receptors of sensitized lymphocytes II. Receptors from nylon wool-enriched rabbit T lymphocytes lack serological determinants of immunoglobulin constant domains but carry a locus allotypic marker. J Exp Med 146:192, 1977.
64. Jensenius JC, Crone M, Koch C. The still elusive T cell receptor. On the possibility of a common V-gene pool for B- and T-cell-antigen receptor molecules. Scand J Immunol 14:693–704, 1981.
64. Owen FL, Riblet R, Taylor BA. The T suppressor cell alloantigen Tsu^d maps near immunoglobulin allotype genes and may be a heavy chain constant-region marker on a T cell receptor. J Exp Med 153:801–810, 1981.
65. Owen FL, Spurll GM. Evidence for a T cell constant region gene family: Characterization of cell surface antigens by immunoprecipitation with alloantisera and monoclonal antibodies. In: Janeway C, Sercarz EE,Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp419–428, 1981.
66. Owen FL, Spurll GM, Panageas E. $Tthy^d$, a new thymocyte alloantigen linked to Igh-1. Implications for a switch mechanism for T cell antigen receptors. J Exp Med 155:52–60, 1982.
67. Kronenberg M, Davis MM, Early PW, Hood LE, Watson JD. Helper and killer T cells do not express B cell immunoglobulin joining and constant region gene segments. J Exp Med 152:1745–1761, 1980.
68. Kurosawa Y, vonBoehmer H, Haas W, Sakarno H, Trauneker A, Tonegawa S. Identification of D segments of immunoglobulin heavy-chain genes and their rearrangement in T lymphocytes. Nature (London) 290:565–570, 1981.
69. Williamson AR. Genes coding for T-lymphocyte receptors. Immunol Today 3:68–72, 1982.
70. Marchalonis JJ, Molecular interactions and recognition specificity of surface receptors. Contemp Topics Immunobiol 9:255–288, 1980.
71. Tada T, Hayakawa K, Okumura K, Taniguchi M. Coexistence of variable region of immunoglobulin heavy chain and I region gene products on antigen-specific suppressor T cells and suppressor T cell factor. A minimal model of functional antigen receptor of T cells. Mol Immunol 17:867–875, 1980.
72. Taussig MJ. Review. Antigen-specific T-cell factors. Immunology 41:759–787, 1980.
73. Robb RJ, Strominger JL, Mann DL. Rapid purification of detergent-solubilized HLA antigen by affinity chromatography employing anti-β_2-microglobulin serum. J Biol Chem 251: 5427–5428, 1976.
74. Zwerner RK, Barstad PA, Acton RT. Isolation and characterization of murine cell surface components. I. Purification of milligram quantities of Thy-1.2. J Exp Med 146:986–1000, 1977.
75. Marchalonis JJ, Hunt JC, Maxwell J, Wang AC. Antigenic polypeptide fragments of a receptor related to the Fab fragment of human immunoglobulin from thymus-derived lymphocytes. Proc Nat Acad Sci USA 79:4733–4736, 1982.
76. Cone RE, Marchalonis JJ, Rolley RT. Lymphocyte membrane dynamics. Metabolic release of cell surface proteins. J Exp Med 134:1373–1384, 1971.
77. Marchalonis JJ, Nossal GJV. Electrophoretic analysis of antibody produced by single cells. Proc Nat Acad Sci USA 61:860–867, 1968.
78. Mage RG. A new look at the biological and genetic significance of rabbit heavy chain allotypes. Ann Immunol (Inst Pasteur) 130C:105–114, 1979.

79. Ben-Neriah Y, Wuilmart C, Lonai P, Givol D. Preparation and characterization of anti-framework antibodies to the heavy chain variable region (V_H) of mouse antibodies. Eur J Immunol 8:797–801, 1978.
80. Rubin B, Hertel-Wulff B, Kimura A. Alloantigen-specific idiotype-bearing receptors on mouse T lymphocytes. I. Specificity characterization and genetic association with the heavy chain IgG allotype. J Exp Med 150:307–321, 1979.
81. Howie S, Feldmann M. *In vitro* studies on H-2-linked unresponsiveness to synthetic polypeptides. III. Production of an antigen-specific T helper cell factor to (T,G)-A—L. Eur J Immunol 7:417–421, 1977.
82. Lonai P, Puri J, Hammerling U. H-2-restricted antigen binding by a hybridoma clone that produces antigen-specific helper factor. Proc Nat Acad Sci USA 78:549–553, 1981.
83. Lonai P, Arman E, Savelkoul HPC, Friedman V, Puri J, Hammerling U. Factors, receptors and their ligands: Studies with H-2 restricted helper hybridoma clones. In: Fathman G, Fitch F, eds. Isolation, Characterization and Utilization of T Lymphocyte Clones. New York, Academic Press, in press.
84. Cone RE, Rosenstein RW, Murray JH, Iverson GM, Ptak W, Gershon RK. Characterization of T-cell surface proteins bound by heterologous antisera to antigen-specific T-cell products. Proc Nat Acad Sci USA 78:6411–6415, 1981.
85. Rosenstein RW, Murray JH, Cone RE, Ptak W, Iverson GM, Gershon RK. Isolation and characterization of an antigen specific, T-cell factor associated with the suppression of DTH. Proc Nat Acad Sci USA, in press.
86. Cone RE, Murray JH, Rosenstein RW, Ptak W, Iversen GM, Gershon RK. The use of heteroantisera to T cell antigen binding proteins as probes for T cell receptors. In: Janeway C, Sercarz EE, Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp457–482, 1981.
87. Fresno M, McVay-Boudreau L, Nabel G, Cantor H. Biologic properties of a purified antigen-specific suppressive glycopeptide. Transplant Proc 13:1124–1127, 1981.
88. Fresno M, McVay-Boudreau L, Nabel G, Cantor RH. Antigen-specific T lymphocyte clones. II. Purification and biological characterization of an antigen-specific suppressive protein synthesized by cloned T cells. J Exp Med 153:1260–1274, 1971.
89. Fresno M, McVay-Boudreau L, Cantor H. Antigen-specific T lymphocyte clones. III. Papain splits purified T suppressor molecules into two functional domains. J Exp Med 155:981–993, 1982.
90. Goodman JW, Lewis GK, Primi D, Hornbeck P, Ruddle NH. Antigen-specific molecules from murine T lymphocytes and T cell hybridomas. Mol Immunol 17:933–945, 1980.
91. Tada T, Nonaka M. T cell hybridomas and their products: An overview. In: Fitch F, Fathman G, eds. Isolation, Characterization and Utilization of T Lymphocyte Clones. New York, Academic Press, in press.
92. Neubauer RH, Marchalonis JJ, Strand BC, Rabin H. Surface markers of primate B and T lymphoid cell lines defined by antibodies to human lymphocyte antigens. J Immunogenet 9: 209–221, 1982.
93. Cramer M, Krawinkel U. Immunochemical properties of isolated hapten-specific T cell receptor molecules. In: Pernis B, Vogel HJ, eds. Regulatory T Lymphocytes. New York, Academic Press, pp39–55, 1980.
94. Moseley JM, Beatty EA, Marchalonis JJ. Molecular properties of T-lymphoma immunoglobulin III. Peptide composition of the light chain. J Immunogenet 6:19–28, 1979.
95. Kontiainen S, Feldmann M. Structural characteristics of antigen-specific suppressor factors: Definition of "constant" region and "variable" region determinants. Thymus 1:59–79, 1979.
96. Tokuhisa T, Taniguchi M. Two distinct allotypic determinants on the antigen-specific suppressor and enhancing T cell factors that are encoded by genes linked to the immunoglobulin heavy chain locus. J Exp Med 155:126–139, 1982.
97. Marchalonis JJ, Wang AC. A marmoset T-lymphocyte protein related to defined human serum immunoglobulin and fragments. J Immunogenet 8:165–175, 1981.
98. Feldmann M, James R, Culbert E, Todd I, Makidono R, Cecka JM, Koniainen S. Uses of antifactor antisera and monoclonals in the analysis of T cell products. In: Janeway C, Sercarz EE, Wigzell H, eds. Immunoglobulin Idiotypes. New York, Academic Press, pp483–492, 1981.
99. Marchalonis JJ, Strelkauskas AJ. A marmoset T lymphoma which functions as a human amplifier T cell. J Immunogen 8:477–483, 1981.
100. Marchalonis JJ, Warr GW, Vasta GR, Ledford BE. Molecular recognition of "non-self" determinants: The existence of a superfamily of recognition molecules related to primordial immunoglobulins. In: Gorczynski RM, ed. Receptors in Biological Systems. New York, Marcel Dekker, in press.
101. Kemp DJ, Harris AW, Cory S, Adams JM. Ex-

pression of the immunoglobulin C_{μ} gene in mouse T and B lymphoid and myeloid cell lines. Proc Nat Acad Sci USA 77:2876–2880, 1980.

102. Kappler JW, Skidmore B, White J, Marrack P. Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. J Exp Med 153:1198–1214, 1981.

103. Lonai P, Bitton S, Savelkoul HFJ, Puri J, Hammerling GJ. Two separate genes regulate self-Ia and carrier recognition in H-2 restricted helper factors secreted by hybridoma cells. J Exp Med, in press.

104. Tada T, Suzuki G, Hiramatsu K. Some comments on the antigen-receptors expressed on functional T cell hybridomas. In: Feldmann M, Schrier MH, eds. Lymphokines, Vol 5. Monoclonal T Cells and Their Products. New York, Academic Press, pp119–128, 1982.

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Suppression of the Natural Killer Cell Activity of Murine Spleen Cell Cultures by Dexamethasone (41489)

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Abstract. Investigations into the mode of action of glucocorticoids on natural killer (NK) cell activity have been hindered by the lack of an *in vitro* model system. We report that the NK activity of spleen cell cultures of several inbred strains of mice was suppressed by treatment with dexamethasone. The *in vitro* suppression of NK activity was time dependent, requiring at least 5 hr incubation in dexamethasone to achieve maximal levels of suppression, and was dose dependent at pharmacologic concentrations. Thus, based on the results of these studies, an *in vitro* model system for studying glucocorticoid effects on NK activity has been established.

Natural killer (NK) cells have been studied extensively in mice, rats, and man (1–4). The ability of these lymphoid cells to lyse many tumor cell lines without prior immunization implicates NK cells as a first line of defense against neoplasia. Recent evidence demonstrating that thymocytes may serve as targets of mouse NK activity suggest an additional role of NK cells in hemopoietic regulation (5). NK cell activity is enhanced by interferon itself or by substances capable of inducing interferon (6–8). In contrast, NK activity is reported to be suppressed *in vivo* by glucocorticoids. Human peripheral NK activity of normal volunteers and patients with systemic lupus erythematosus is depressed by glucocorticoid treatment (9, 10). Similarly, rat and mouse NK activity is impaired severely 12 to 24 hr after hydrocortisone administration (11, 12). It is not known whether glucocorticoids act directly or indirectly on NK cells and the lack of an *in vitro* model system has hampered such investigations. Although Hochman and Cudkowicz attribute suppressed NK activity in mice after hydrocortisone treatment to stimulation of suppressor cells, more recent evidence demonstrated that the spleen cells of cortisone acetate-treated mice, having depressed NK activity, failed to suppress the NK activity of normal mice (13, 14). To understand the mechanism underlying glucocorticoid action on NK activity, the establishment of an *in vitro* model system is essential. We report here the *in vitro* suppression of NK cell activity of mouse spleen cell cultures by pharmacologic concentrations of dexamethasone.

Materials and Methods. *Mice.* Inbred A/J (H-2^a), BALB/cJ (H-2^d), CBA/J (H-2^k), C3H/HeJ (H-2^k), C57Bl/6J (H-2^b), and DBA/2J (H-2^d) mice were purchased from Jackson Laboratories (Bar Harbor, Maine). All mice used in these studies were males between 5 and 12 weeks of age.

Target cells. YAC-1 lymphoma cells, previously described (15), were maintained in RPMI 1640 (Flow Laboratories, Rockville, Md.) medium supplemented with 10% fetal bovine serum (FLOW), 2 m*M* L-glutamine (Grand Island Biological Co., Grand Island, N.Y.), 100 U/ml penicillin, and 100 μg/ml streptomycin (GIBCO).

Steroid treatment. Dexamethasone (Merck, Sharp and Dohme, West Point, Pa.) was initially prepared as a 10^{-4} *M* stock solution in 95% ethanol and stored at 4°. For use in spleen cell cultures, 0.5 ml of the

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stock solution was evaporated in a 60 × 15-mm plastic tissue culture dish (Falcon, Oxnard, Calif.). The dexamethasone was redissolved in 5.0 ml of complete RPMI 1640 (RPMI supplemented with 10% FBS, 2 m*M* L-glutamine, 15 m*M* HEPES, 5×10^{-5} *M* 2-Mercaptoethanol, and antibiotics) by incubation at 37° for 30 min. Control medium was prepared in the same manner except that dexamethasone was omitted from the ethanol. Thus, both control and steroid preparations were virtually ethanol free. The pooled spleen cells of three to five mice were incubated at 37°, 5% CO_2 in RPMI 1640 containing dexamethasone or control medium. The cells were then washed twice in ice-cold RPMI 1640, counted in trypan blue with a hemacytometer, and adjusted to 10^7 viable cells/ml.

Cytotoxicity assay. Different effector to target cell ratios were prepared by twofold serial dilutions in complete RPMI 1640. NK activity was measured by a modification of the ^{51}Cr release assay (16). Briefly, 0.1 ml of YAC-1 target cells radiolabeled by incubation in $Na_2{}^{51}CrO_4$ (New England Nuclear, Cambridge, Mass.) were added to the individual wells of 96-well microtiter plates (Nunclon, Roskilde, Denmark) containing 0.1 ml of spleen cell suspension. The plates were centrifuged at 250*g* for 2 min and then incubated for 4 hr at 37° in a humidified atmosphere of 95% air, 5% CO_2. The assays were terminated by centrifugation at 500*g* for 10 min. Released radioactivity in 0.1 ml of the supernatant fluid was measured by liquid scintillation spectrometry. The percentage cytotoxicity was computed from

$$\% \text{ cytotoxicity} = (\text{cpm}_{exp} - \text{cpm}_{sr})/(\text{cpm}_{max} - \text{cpm}_{sr}) \times 100.$$

cpm_{exp}, cpm_{max}, and cpm_{sr} represent, respectively, the counts per minute in supernatants from YAC-1 target cells incubated with effector cells, from target cells lysed with 5% sodium dodecyl sulfate, and from target cells incubated without effector cells to give a measure of spontaneous release. Typically, spontaneous isotope release from target cells was less than 4% of the maximum isotope incorporation. Except where indicated, all results are expressed as the mean percentage cytotoxicity of at least three replicate cultures of one of several experiments. Standard deviations of means of the experiments shown were less than 2% unless depicted. Three effector to target cell ratios, 25:1, 50:1, and 100:1 were used in all experiments and gave consistent results.

Experimental Results. *Kinetics of suppression of NK activity by dexamethasone.* Mouse NK activity was measured in 5-hr chromium-51 release assays after preincubation of whole spleen cell preparations in dexamethasone, a potent synthetic glucocorticoid. Cultures that received control medium demonstrated 10 to 11% cytotoxicity. In contrast, NK activity in 10^{-7} *M* dexamethasone-treated cultures decreased from 11 to 9% cytotoxicity after 1 hr of incubation in medium containing the glucocorticoid (Fig. 1). By 6 hr of exposure

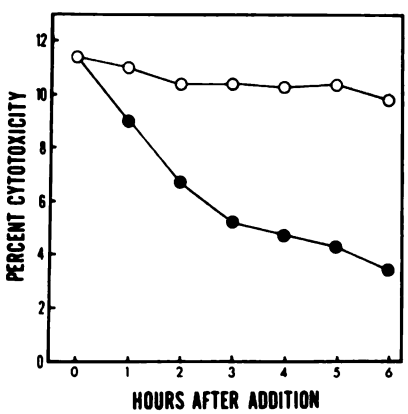

FIG. 1. Kinetics of suppression of mouse NK activity after *in vitro* cultivation in dexamethasone. C57Bl/6 spleen cells were incubated in conical centrifuge tubes at 37°, 5% CO_2. At hourly intervals, control medium (○) or dexamethasone (●) was added to the appropriate cultures in volumes sufficient to yield final concentrations of 10^{-7} *M* dexamethasone. After a total of 6 hr incubation, the spleen cells were washed and assessed for NK activity against ^{51}Cr-labeled YAC-1 cells. Three effector to target cell ratios were used and gave consistent results. Shown here are the results for the 100:1 ratio of a representative experiment. Standard deviations of the means of each point were less than 2% cytotoxicity.

to dexamethasone, NK activity had declined to 3% cytotoxicity, about one-third of the activity in control cultures. In three such experiments, the NK activity in cultures treated for 6 hr with 10^{-7} *M* dexamethasone was significantly decreased compared to the NK activity of control cultures ($P < 0.001$, Student's *t* test).

Experiments in which inhibitory concentrations of dexamethasone were added directly into the cytotoxicity assay wells or in which YAC-1 target cells were cultured for 24 hr prior to assay in medium containing 10^{-7} *M* dexamethasone failed to demonstrate decreased levels of NK mediated cytotoxicity (data not shown). Thus, the suppression of NK activity in dexamethasone-treated cultures is not attributable to the increased resistance of target cells to lysis after contamination of the cytotoxicity assays by possible carryover of dexamethasone from the preincubation cultures, but to a dexamethasone effect on the spleen cells.

Dexamethasone dose response. In order to assess the dose response relationship of the dexamethasone-induced *in vitro* suppression of NK cell activity, unfractionated spleen cells were incubated for 5 hr in various concentrations of dexamethasone before assessment of NK cytotoxicity. The results of a representative experiment shown in Table I clearly demonstrate that incubation of unfractionated spleen cells in concentrations as low as 10^{-9} *M* dexamethasone was sufficient to induce dramatic decreases in target cell killing. Suppression of NK cytotoxicity was dose dependent from 10^{-11} *M* to 10^{-8} *M* dexamethasone. Increasing the concentration of dexamethasone to 10^{-7} *M* or 10^{-6} *M* did not increase the degree of suppression of NK activity. Thus, *in vitro* suppression of NK activity by dexamethasone is dose dependent and occurs at pharmacologic concentrations. Similar results also were obtained from spleen cell suspensions of adrenalectomized mice (data not shown).

Dexamethasone suppression of NK activity of different mouse strains. The NK activity of inbred mice of different genetic backgrounds was tested for sensitivity to *in vitro* dexamethasone treatment. The results of a representative experiment, Fig. 2, demonstrate that the NK activity of all but one strain tested was strikingly suppressed after 5 hr incubation in medium containing 10^{-7} *M* dexamethasone. The exception, the spleen cells of A/J mice, had very little NK activity. Consequently, the suppressive effect of dexamethasone may not have been as apparent as that seen in the other strains that expressed much higher levels of NK activity. Nonetheless, it is clear that *in vitro* dexamethasone treatment suppresses

TABLE I. DOSE-DEPENDENT *IN VITRO* SUPPRESSION OF NK ACTIVITY BY DEXAMETHASONE

	Percentage cytotoxicity		
DEX (M)[a]	25[b]	50	100
0	6.1	9.4	12.7
10^{-11}	5.9	8.4	13.2
10^{-10}	4.4	7.0	10.2
10^{-9}	1.4	3.4	4.6
10^{-8}	1.4	2.0	3.1
10^{-7}	0.8	1.5	2.0
10^{-6}	0.8	1.4	2.5

[a] C57BL/6J spleen cells (10^7/ml) were incubated 5 hr in complete RPMI 1640 containing the indicated concentrations of dexamethasone, washed, and assayed for NK activity against YAC-1 target cells.

[b] Effector cell to target cell ratio.

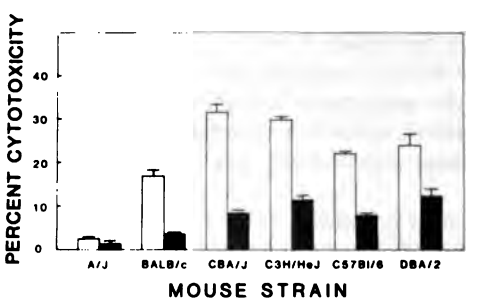

FIG. 2. Dexamethasone-induced suppression of NK activity of various mouse strains. The spleen cells of inbred mice of different genetic backgrounds were incubated 5 hr in control medium (□) or medium containing 10^{-7} *M* dexamethasone (■). After washing, the NK activity of each culture was determined against YAC-1 cells. Although tested at three different effector to target cell ratios, only the mean percentage cytotoxicity (±SD) of the 100:1 cultures are shown in this representative experiment.

murine NK activity regardless of genetic background.

The results of this study establish an *in vitro* model system for investigating the action of glucocorticoids on NK activity. The reduction of NK activity in mouse spleen cell cultures after dexamethasone treatment is time dependent and dose dependent at pharmacologic concentrations. Since the proportion of cells possessing NK activity is thought to be less than 5% of the total splenic population, viability studies are of little value in determining whether DEX is toxic for NK effector cells (17). Nonetheless, the viabilities of treated and control cultures are similar. Additional studies employing this *in vitro* system do not demonstrate the presence of suppressor cell activity after DEX treatment although NK activity is depressed (manuscript in preparation). This would suggest that glucocorticoids suppress NK activity by acting directly on the NK effector cell.

In vitro suppression of NK activity by meaningful concentrations of glucocorticoids, to our knowledge, has not been previously demonstrated although Parillo and Fauci reported that human NK activity is decreased by the addition of very high concentrations of DEX (10^{-5} *M* and 10^{-4} *M*, termed "pharmacologic" and "supraphar-macologic") to 18-hr cytotoxicity assays (9). Since these concentrations are known to produce nonspecific effects, the relevance of these results are questionable (18).

We have obtained very similar *in vitro* glucocorticoid effects on human NK activity (submitted for publication). Incubation of Ficoll–Hypaque-enriched mononuclear cells of normal volunteers for 24 hr in 5×10^{-7} *M* dexamethasone suppressed the NK activity to only 30% of the activity in cultures that had received control medium. Thus, the *in vitro* model system we describe here appears to closely simulate not only the *in vivo* glucocorticoid-induced suppression of NK cytotoxicity in mice but also in humans.

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1. Herberman RB, Nunn MF, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. Int J Cancer 16:230–239, 1975.
2. Kiessling RE, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol 5:117–121, 1975.
3. Oehler JR, Lindsay LR, Nunn ME, Herberman RB. Natural cell-mediated cytotoxicity in rats. I. Tissue and strain distribution, and demonstration of a membrane receptor for the Fc portion of IgG. Int J Cancer 21:204–209, 1978.
4. Jondal M, Pross H. Surface markers on human B and T lymphocytes. VI. Cytotoxicity against cell lines as a functional marker for lymphocyte subpopulations. Int J Cancer 15:596–605, 1975.
5. Hansson M, Kärre K, Kiessling R, Roder J, Andersson B, Häyry P. Natural NK-cell targets in the mouse thymus: Characteristics of the sensitive cell population. J Immunol 123:765–771, 1979.
6. Gidlund M, Orn A, Wigzell H, Senik A, Gresser I. Enhanced NK cell activity in mice injected with interferon and interferon inducers. Nature (London) 273:759–761, 1978.
7. Herberman RB, Djeu JY, Ortaldo JR, Holden HT, West WH, Bonnard GD. Role of interferon in augmentation of natural and antibody-dependent cell-mediated cytotoxicity. Cancer Treatment Rep 62:1893–1896, 1978.
8. Senik A, Gresser I, Maury C, Gidlund M, Örn A, Wigzell H. Enhancement of mouse NK cells by interferon. Transplant Proc 11:993–996, 1979.
9. Parillo JE, Fauci AS. Comparison of the effector cells in human spontaneous cellular cytotoxicity and antibody-dependent cellular cytotoxicity: Differential sensitivity of effector cells to in vivo and in vitro corticosteroids. Scand J Immunol 8:99–107, 1978.
10. Oshiani K, Gonda M, Sumiya M, Kano S. Effects of corticosteroids on natural killer cell activity in systemic lupus erythematosus. Clin Exp Immunol 40:83–88, 1980.
11. Shellam GR. Gross-virus-induced lymphoma in the rat. V. Natural cytotoxic cells are non-T-cells. Int J Cancer 19:225–235, 1977.
12. Hochman PS, Cudkowicz G. Different sensitivities to hydrocortisone of natural killer cell activity and hybrid resistance to parental marrow grafts. J Immunol 119:2013–2015, 1977.
13. Hochman PS, Cudkowicz G. Suppression of natu-

ral cytotoxicity by spleen cells of hydrocortisone-treated mice. J Immunol 123:968–976, 1979.

14. Lotzova E, Savary GA. Parallelism between the effect of cortisone acetate on hybrid resistance and natural killing. Exp Hematol 9:766–774, 1981.
15. Klein G, Klein E, Haughton GJ. Variation of antigenic characteristics between different mouse lymphomas induced by the Moloney virus. J Natl Cancer Inst 36:607–621, 1966.
16. Brunner KT, Mauel J, Cerotinni JC, Chapuis B. Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labelled allogeneic target cells *in vitro;* inhibition by isoantibody and by drugs. Immunology 14:181–196, 1968.
17. Roder JC, Kiessling R. Target–effector interaction in the natural killer cell system. I. Covariance and genetic control of cytolytic and target-cell-binding subpopulation in the mouse. Scand J Immunol 8:135–144, 1978.
18. Munck A. Steroid concentration and tissue integrity as factors determining the physiological significance of effects of adrenal steroids *in vitro*. Endocrinology 77:356–360, 1965.

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Macromegakaryocytosis After Hydroxyurea[1] (41490)

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Abstract. A single injection of hydroxyurea (OHU) produced transient megakaryocytopenia in mice. An increase in the average mean size of mature, stage III megakaryocytes coincided with their depopulation. This was due to a selective reduction in numbers of smaller cells. In contrast, the macromegakaryocytosis of immunothrombocytopenia showed substantial increases in numbers of larger cells and reductions in smaller. Further reduction in numbers of smaller cells occurred when OHU was given to mice with immunothrombocytopenia, and the megakaryocytopenia was somewhat more severe than that produced by OHU in normal mice. OHU produced mild thrombocytopenia in normal mice and compromised recovery of the platelet count from immunothrombocytopenia. The most likely explanation for the increase in mean megakaryocyte size in the hypomegakaryocytic state produced by OHU is that the temporary imbalance between a low rate of influx and a normal rate of maturation produced a shift of the age distribution of the cells due to a deficiency of immature cells.

Megakaryocyte size adjusts in response to perturbations of platelet count, demonstrating that it is one of the variables in the regulation of platelet production (1, 2). When megakaryocytopoiesis is stimulated or suppressed by perturbations of the platelet count, ploidy and size of megakaryocytes are, respectively, increased or decreased (2, 3). Size is determined not only by ploidy but also by the level of maturation in the compartment of recognizable megakaryocytes (4). Within any maturation stage, megakaryocyte size is proportional to ploidy, and within any ploidy group, size is proportional to maturity (5, 6). Megakaryocyte maturation is subject to some degree of variability being accelerated in response to thrombocytopenia (4) but remaining normal in transfusion-induced thrombocytosis (7). However, instances in which changes in the age distribution of megakaryocytes alone may have effected changes in mean cell size have not been described.

In addition to the megakaryocyte size changes that can be produced by manipulation of the platelet count, mean megakaryocyte size may be increased in hypomegakaryocytic states in which thrombocytopenia does not appear to be causative (8). The present studies were done to analyze the macromegakaryocytosis that accompanies the transient hypomegakaryocytic state following a single injection of hydroxyurea (OHU).

Materials and Methods. Female mice of the CF_1 strain (Charles River) were used at the age of 12–14 weeks. Each mouse was sampled only once; sequential studies were done with cohorts of mice. Blood for platelet counts was obtained by cardiac puncture under ether anesthesia and anticoagulated with dry K_2EDTA; platelets were counted by phase microscopy (9). Cells were flushed out from each tibia with 1 ml of 1% Na_2EDTA in saline for counts of nucleated cells by Coulter counter and megakaryocytes by microscopy with new methylene blue stain.

Bone marrow smears were made from split femurs with a paint brush technique

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and stained with Wright's and Giemsa stains. Megakaryocytes were classified according to morphological criteria; all megakaryocytes were larger than myeloid and erythroid cells. Stage I megakaryocytes had basophilic cytoplasm without visible granules and a high nucleus/cytoplasm ratio. Stage III had azurophilic granules throughout the cytoplasm and a low nucleus/cytoplasm ratio. Stage II had intermediate morphology. Differential counts were done to classify 100 megakaryocytes from each mouse as stage I, II, or III. Sizes of stages I and III megakaryocytes were determined by measuring the areas of images enlarged from negatives of black and white photomicrographs; the areas were expressed as planimeter units.

Anti-mouse platelet serum (APS) was produced in rabbits or guinea pigs; before use it was heat inactivated and absorbed three times with equal volumes of washed mouse red cells. It was injected ip in 0.1 ml volumes after appropriate dilution with saline.

OHU was freshly dissolved in saline before injection. It was injected iv (tail vein) in a dose of 900 mg/kg body weight.

Results. One day after a single injection of OHU, tibial megakaryocytes and total nucleated cells declined to about 80% of normal (Fig. 1). Megakaryocytes decreased further to about half of normal on Days 2 and 3 before beginning to recover on Day 4. Total cellularity was less severely diminished and recovered about one day sooner than megakaryocytes. Cell counts returned to, but did not exceed, normal numbers during the week following administration of OHU.

A plot of average stage III megakaryocyte size (Fig. 2) was a mirror image of the plot of megakaryocyte numbers; size increased as number decreased, and both returned to normal at the same time. The maximum increase in mean size of stage III megakaryocytes occurred on Days 2 and 3, and it was not preceded by an increase in mean size of stage I cells on Day 1. This finding confirmed earlier unpublished observations in which rats were given 900 mg OHU/kg; for 3 days there was a gradual increase in average size of stage III cells to about 140% of normal with no change in average size of stage I cells.

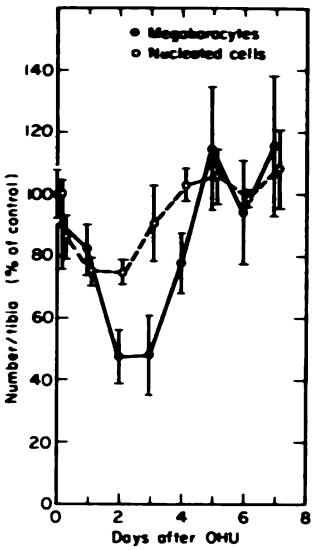

FIG. 1. Numbers of tibial megakaryocytes and nucleated cells, expressed as percentage of control values, for 7 days after administration of OHU to mice. Mean ± SEM for 33 controls is shown at 0 time; other points represent 11–12 mice.

To evaluate the changes in mean size of stage III cells, it was necessary to determine the relative number of stage IIIs at each sampling time after OHU. Differential counts of stages I, II, and III were done (Fig. 3, left); multiplication of percentage of each by the relative total number of megakaryocytes (Fig. 1; controls = 100) yielded the relative number of each stage in the marrow (Fig. 3, right). Megakaryocyte depletion occurred first in stage I cells and progressed to involve all three stages. Normal numbers of stage III cells were maintained through the first post-treatment day after which they decreased. On Days 2 and 3, the 50% reduction in total megakaryocytes was associated with a normal differential count, and therefore, equal reduction in all stages.

Size distribution curves for stage III megakaryocytes were prepared by determining the percentage of the cells that were in each 500-unit segment of the size range

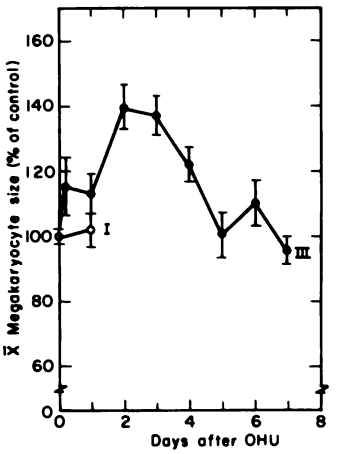

FIG. 2. Mean megakaryocyte size (±SEM), expressed as percentage of control values, after administration of OHU to mice. Stage III control value represents 1087 megakaryocytes from 33 mice; subsequent points, 376–386 megakaryocytes from 12 mice. Stage I control value represents 474 megakaryocytes from 33 mice and, at one day, 118 megakaryocytes from 12 mice.

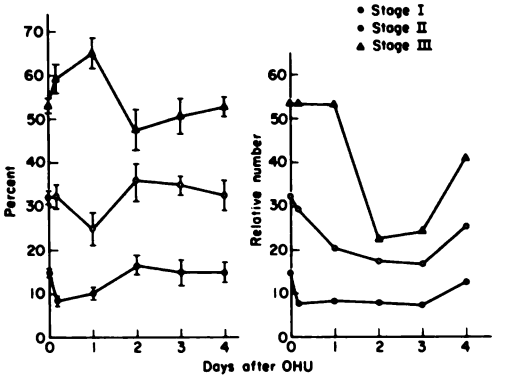

FIG. 3. Left panel: differential count of stages I, II, and III megakaryocytes after administration of OHU to mice; 100 megakaryocytes from each of 33 controls and 12 mice at each time after OHU were classified. Results are means ± SEM. Right panel: relative number of each maturation stage was calculated by multiplying mean percentage (left panel) by relative mean total number of megakaryocytes (Fig. 1).

(0–4000 units) at each sampling time. Each percentage was then multiplied by the relative number of stage III's at the same sampling time to develop size distribution curves that were representative of the actual number of stage III megakaryocytes and thus accounted for changing numbers of cells (Fig. 4). The 40% increase in mean size on Days 2 and 3 was due to a disproportionately great reduction in numbers of smaller megakaryocytes (<1000 units) and retention of normal numbers of larger cells (>1250 units); intermediate cells were diminished in proportion to the whole stage III population. Partial recovery on Day 4 was due to influx of intermediate size cells rather than those at either extreme of the curve.

Platelet counts after OHU are shown in Fig. 5; they were normal for 3 days, but declined to about 85% of normal on Days 4 and 5.

The effects of OHU on APS-treated mice were similar when the OHU was given after 1 day or after 4 days of APS-induced thrombocytopenia, so only the results of OHU after 1 day of thrombocytopenia are presented. Because of variability in platelet and megakaryocyte counts, two experiments are presented individually; however, tibial cell counts were almost identical in all four experiments done with APS and OHU, thus indicating that there was a consistent response to the chemical in all experiments.

Recovery from APS-induced thrombocytopenia was associated with variable degrees of rebound thrombocytosis and increases in numbers of tibial megakaryocytes, but there was a consistent increase in mean size of stage III megakaryocytes (Fig. 6). Administration of OHU retarded recovery of platelet counts and prevented rebound thrombocytosis. Maximal reductions in megakaryocyte numbers occurred 2 days after administration of OHU and were about one-third to one-half of the concomitant values in mice treated only with APS. Mean stage III megakaryocyte size was greater after combined treatment with APS and OHU than after APS alone, and the first appearance of a difference in size coincided with the reduction in number.

Size distributions of stage III megakaryocytes for the Day 2 samples were corrected for changing numbers of cells from the differential count of stages I, II, and III and total numbers of megakaryocytes

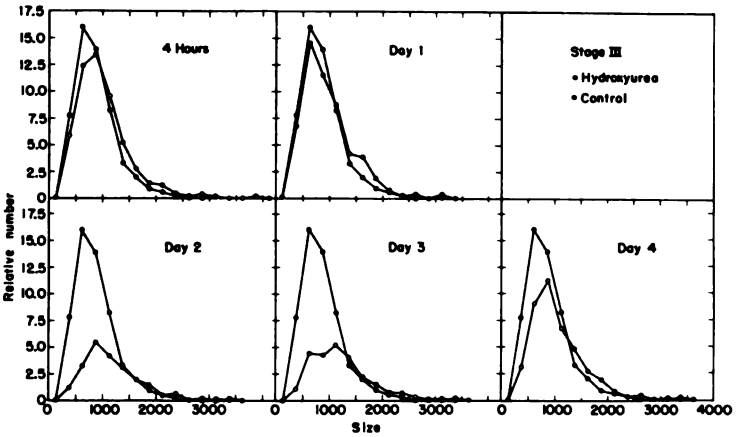

FIG. 4. Size distribution curves for stage III megakaryocytes from control mice and at intervals after administration of OHU. Abscissa expresses size in arbitrary units; ordinate is proportional to actual numbers of cells per tibia. Controls represent 1087 megakaryocytes from 33 mice; each curve after OHU represents 376–386 megakaryocytes from 12 mice.

as described above. Size distribution curves which are, therefore, representative of the actual numbers of stage III megakaryocytes are presented for the two experiments in Fig. 7. APS-induced thrombocytopenia alone was associated with reduced numbers of smaller megakaryocytes (<1000 units), substantial increases in larger ones (>1250 units), and inconsistent appearance of a few cells larger than any normally found in the marrow. OHU had the same effect on the size

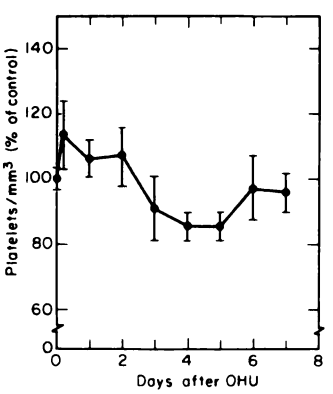

FIG. 5. Platelet counts, expressed as percentage of control, of 33 control mice and 11–12 mice at each sampling time after administration of OHU. Each point is the mean ± SEM.

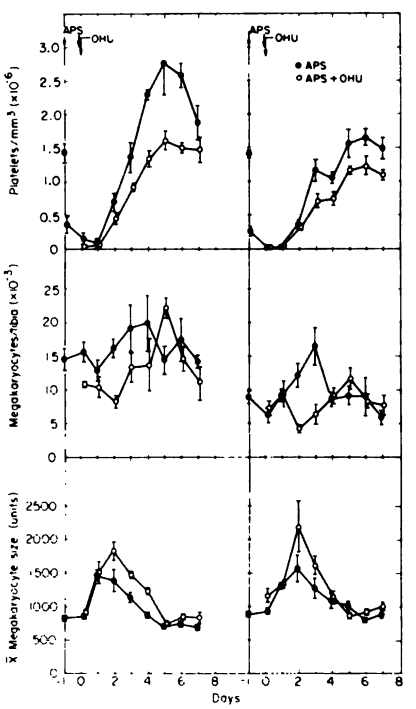

FIG. 6. Platelet counts, tibial megakaryocytes, and mean stage III megakaryocyte sizes in mice given APS on Days −1 and 0 with or without OHU on Day 0. Two experiments are shown. Each control is shown at 0 time and represents eight mice; each other point represents three to five mice. Each point is the mean ± SEM.

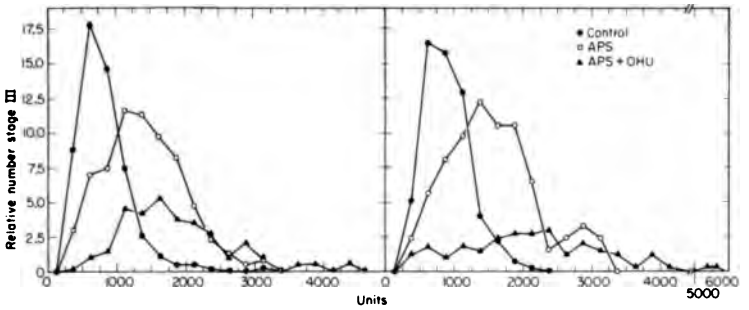

FIG. 7. Size distribution curves for stage III megakaryocytes from the same mice depicted in Fig. 6. Abscissa is size units; ordinate is proportional to actual numbers of cells per tibia. Controls represent 289 and 160 megakaryocytes from seven and eight mice. APS treatment represents 174 and 94 megakaryocytes from five mice in each experiment. APS + OHU treatment represents 175 and 88 megakaryocytes from five mice in each experiment. Values for treated mice were obtained 2 days after the treatment.

distribution of megakaryocytes in APS-treated mice as it had had in normals, i.e., retention of the largest cells, reduction in number of intermediate cells proportional to the reduction in the whole population, and disproportionately great reduction in numbers of smaller cells.

Discussion. The present results help to explain how macromegakaryocytosis develops in the hypomegakaryocytic state produced by OHU (8).

The number of stage I megakaryocytes was reduced as early as 4 hr after administration of OHU, indicating that the drug destroyed either stage I cells directly or precursors that should have matured rapidly to become stage I's. Long *et al.* (10) concluded that stage I megakaryocytes are actually a part of a population of smaller cells with lobed nuclei that could be identified histochemically as megakaryocytes. They did not find a cytocidal effect of OHU on this population 3 hr after its injection; rather, they found a 57% reduction in small precursor cells with round nuclei. Transit time for stage I rat and mouse megakaryocytes has been estimated to be 6–14 hr (11–13). Thus, the drop of about 50% at 4 hr could have been due to cessation of influx from a drug-sensitive compartment, but it seems more likely that some of the stage I cells were directly killed by OHU in these experiments.

The deficiency of megakaryocytes was apparent in stages II and III 1 to 3 days after administration of OHU as the requisite time elapsed for the damaged populations to mature into those compartments. Persistence of low numbers of megakaryocytes for 3 days indicated that OHU destroyed cells in an important precursor compartment, probably a dividing 2N cell population. The delay in recovery could not be attributed to prolonged action of the drug, as Morse *et al.* (14) have shown that DNA synthesis resumes as soon as 2 hr after 900 mg OHU/kg in the bone marrow of CF_1 mice.

Depopulation of the stage III compartment was associated with an increase in the mean cell size, due to selective loss of smaller stage III's without an increase in numbers of larger cells. Thus it contrasted with the macromegakaryocytosis produced in response to immunothrombocytopenia in which larger megakaryocytes were increased in number at the apparent expense of smaller ones. The macromegakaryocytosis produced in response to peripheral thrombocytopenia is known to be associated with increased ploidy (2, 3). Endoreduplication is completed and final ploidy determined before the cells leave stage I (11, 12, 15), and macrocytosis of stage I cells precedes that of stage III cells when stimulated by platelet depletion (1). Failure of stage I's to

show increases in size after OHU shows that the mechanism responsible for stage III macrocytosis after OHU differs from that seen after thrombocytopenia.

Megakaryocytes increase progressively in size as they mature from stage I through stage III (1); since stage III occupies 1–2 days of the 2- to 3-day total transit time (12, 13), it can be assumed that they continue to grow within this morphological group. Reduced influx in the presence of a normal transit time would account for the presence of fewer immature, and smaller stage III's during the period when their number was dropping. The somewhat greater reduction in megakaryocytes produced by OHU in APS-stimulated mice than in normals could then be attributed to the shorter transit time that occurs in thrombocytopenic animals (4, 16). The uniqueness of the OHU-treated mice is the increase in mean megakaryocyte size while their number is dropping. The mere reduction in smaller megakaryocytes has been observed (17) in irradiated mice during periods of stable megakaryocyte counts in which, therefore, an imbalance between rate of influx and rate of maturation does not appear to be involved.

Levin *et al.* (18) and Paulus *et al.* (19) have observed an inverse relationship between numbers of cells and their ploidy in megakaryocyte colonies cultured from hemopoietic cells. In these *in vitro* systems, therefore, the more a precursor cell divides, the less it appears to endoreduplicate. If this principle applies *in vivo*, the precursors of the smaller stage III's might have divided more than the precursors of larger ones and, therefore, have been more heavily damaged by OHU. This notion, however, would imply that the larger cells that persisted after OHU would have a higher degree of polyploidy than those that disappeared, and, as noted above, the failure of stage I cells to enlarge speaks against there being significant shifts in ploidy distribution.

OHU produced minor changes in platelet counts, probably because of the transient nature of the reduction in megakaryocytes. Considering that mouse platelets survive for about 4 days (20), the 15% drop in platelet count on Day 4 is consistent with a platelet production rate of about 50% for the precedent 24 hr, which, in turn, is consistent with the megakaryocyte number having been 50% of normal on Day 3. However, platelet counts on Days 3 and 4 were somewhat greater than would have been expected if production had been reduced to 50% for 2 days as might have been expected from the numbers of stage III megakaryocytes. It could be proposed that, even though total cell number was reduced on Day 2, those that remained represented a nearly normal complement of the most mature, platelet-forming, stage III cells.

Recovery from immunothrombocytopenia was delayed, and rebound thrombocytosis was prevented, demonstrating a somewhat different effect for OHU than was seen when vincristine (VC) was administered to acutely or chronically thrombocytopenic rats (21). VC inhibited recovery from acute, but not chronic, thrombocytopenia whereas OHU inhibited recovery from both. The two drugs produced comparable reduction in megakaryocytes, so the reason for the difference is not clear. However, a difference in ability to recover from acute immunothrombocytopenia has also been found between genetically anemic mice of the W/W^v (22) and Sl/Sl^d (23) strains. Both strains are comparably hypomegakaryocytic, but the W/W^v responds normally to acute platelet depletion whereas the Sl/Sl^d fails to develop rebound thrombocytosis. Neither the drugs nor the genetic abnormalities interfered with the development of macromegakaryocytosis. These variable responses to thrombocytopoietic stimulation under different conditions indicate that platelet production can not be accurately predicted from just the number of megakaryocytes and their mean size and that other factors must also be important.

1. Ebbe S, Stohlman F Jr, Overcash J, Donovan J, Howard D. Megakaryocyte size in thrombocytopenic and normal rats. Blood 32:383–392, 1968.
2. Odell TT, Murphy JR, Jackson CW. Stimulation of megakaryocytopoiesis by acute thrombocytopenia in rats. Blood 48:765–775, 1976.
3. Penington DG, Olsen TE. Megakaryocytes in states of altered platelet production: Cell numbers,

size and DNA content. Brit J Haematol 18:447–463, 1970.
4. Ebbe S, Stohlman F Jr, Donovan J, Overcash J. Megakaryocyte maturation rate in thrombocytopenic rats. Blood 32:787–795, 1968.
5. Odell TT Jr, Jackson CW. Polyploidy and maturation of rat megakaryocytes. Blood 32:102–110, 1968.
6. Odell TT Jr, Jackson CW, Friday TJ. Megakaryocytopoiesis in rats with special reference to polyploidy. Blood 35:775–782, 1970.
7. Ebbe S, Stohlman F Jr, Donovan J, Howard D. Megakaryocytopoiesis in the rat with transfusion-induced thrombocytosis. Proc Soc Exp Biol Med 122:1053–1057, 1966.
8. Ebbe S, Phalen E. Does autoregulation of megakaryocytopoiesis occur? Blood Cells 5:123–138, 1979.
9. Brecher G, Cronkite EP. Morphology and enumeration of human blood platelets. J Appl Physiol 3:365–377, 1950.
10. Long MW, Williams N, Ebbe S. Immature megakaryocytes in the mouse: Physical characteristics, cell cycle status, and in vitro responsiveness to thrombopoietic stimulatory factor. Blood 59:569–575, 1982.
11. Feinendegen LE, Odartchenko N, Cottier H, Bond VP. Kinetics of megakaryocyte proliferation. Proc Soc Exp Biol Med 111:177–182, 1962.
12. Ebbe S, Stohlman F Jr. Megakaryocytopoiesis in the rat. Blood 26:20–35, 1965.
13. Ebbe S. The megakaryocyte: maturation and self-renewal. In: Brinkhous KM, Shermer RW, Mostofi FK, eds. The Platelet. Baltimore, Williams & Wilkins, p1, 1971.
14. Morse BS, Rencricca NJ, Stohlman F Jr. The effect of hydroxyurea on differentiated marrow erythroid precursors. Proc Soc Exp Biol Med 130:986–989, 1969.
15. Odell TT Jr, Jackson CW, Gosslee DG. Maturation of rat megakaryocytes studied by microspectrophotometric measurement of DNA. Proc Soc Exp Biol Med 119:1194–1199, 1965.
16. Odell TT Jr, Jackson CW, Friday TJ, Charsha DE. Effects of thrombocytopenia on megakaryocytopoiesis. Brit J Haematol 17:90–101, 1969.
17. Ebbe S, Phalen E, Threatte G, Adrados C. Megakaryocytopoiesis in irradiated, splenectomized mice. Exp Hematol 9:1020–1027, 1981.
18. Levin J, Levin FC, Penington DG, Metcalf D. Measurement of ploidy distribution in megakaryocyte colonies obtained from culture: With studies of the effects of thrombocytopenia. Blood 57:287–297, 1981.
19. Paulus JM, Prenant M, Maigne J, Henry-Amar M, Deschamps JF. Ploidization of megakaryocyte progenitors *in vitro*. In: Evatt BL, Levine RF, Williams NT, eds. Megakaryocyte Biology and Precursors: In Vitro Cloning and Cellular Properties. New York, Elsevier/North-Holland, p171, 1981.
20. Odell TT Jr, McDonald TP. Life span of mouse blood platelets. Proc Soc Exp Biol Med 106:107–108, 1961.
21. Ebbe S, Howard D, Phalen E, Stohlman F Jr. Effects of vincristine on normal and stimulated megakaryocytopoiesis in the rat. Brit J Haematol 29:593–603, 1975.
22. Ebbe S, Phalen E. Regulation of megakaryocytes in W/W^v mice. J Cell Physiol 96:73–80, 1978.
23. Ebbe S, Phalen E, D'Amore P, Howard D. Megakaryocytic responses to thrombocytopenia and thrombocytosis in Sl/Sl^d mice. Exp Hematol 6:201–212, 1978.

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Regional Uptake of [^{3}H]Norepinephrine by the Canine Left Ventricle[1] (41491)

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Abstract. The distribution of sympathetic neurons within the canine left ventricle was assessed by measuring the myocardial uptake of [^{3}H]norepinephrine in anesthetized dogs. The nonneuronal uptake of [^{3}H]norepinephrine was also assessed in a separate group of cocaine-treated animals. The left ventricle was systematically divided into multiple sections. The [^{3}H]norepinephrine was isolated in tissue homogenates, using alumina extraction, and quantified by liquid scintillation counting. The results revealed a nonuniform pattern of [^{3}H]norepinephrine uptake in the ventricular free wall, with a greater uptake in the base than in the apex. Differences in uptake between the anterior and posterior regions of the free wall, and between the free wall and the septum were not statistically significant. In the cocaine-treated animals uptake was approximately 20% of that in the control animals. Furthermore, there were no significant regional differences. These data suggest that in the canine left ventricle the sympathetic nerves are distributed nonuniformly between the base and apex, but otherwise the distribution is uniform.

Although neural control of the heart is a topic of considerable current interest (1, 2), very little information is available on the regional distribution of cardiac sympathetic neurons within the left ventricular myocardium. The sympathetic nerve supply to the left ventricle has been shown to cross from the pulmonary artery-aortic region to the ventricle and to course in the epicardium in a base to apex direction before branching and innervating the deeper layers of the myocardium (3–5). In previous studies concerned with regional innervation of the canine left ventricle, Angelakos (6) found a difference in norepinephrine content between the base and the apex, and Dahlström *et al.* (7) obtained histological evidence of nonuniform transmural sympathetic innervation.

The purpose of the present study was to characterize further the regional sympathetic innervation of the canine left ventricle. To accomplish this we utilized the [^{3}H]norepinephrine uptake method of Kaye and Tyce (8), in which [^{3}H]norepinephrine uptake has been shown to be a reliable index of cardiac sympathetic innervation. Results were obtained in both normal and cocaine-treated animals to distinguish between the neuronal and nonneuronal uptake of [^{3}H]norepinephrine in different regions of the ventricle.

Methods. The experiments were performed on 13 male mongrel dogs (20–40 kg) who had been maintained on a nourishing diet for at least 30 days. The animals were fasted overnight, premedicated with morphine sulfate (2.5 mg/kg, sc), and anesthetized 45–60 min later with α-chloralose (100 mg/kg, iv). Supplemental doses of α-chloralose were given as required prior to the administration of [^{3}H]norepinephrine, but additional anesthetic was avoided thereafter. The trachea was intubated with a cuffed endotracheal tube and the animal was ventilated with a Harvard respirator (Model 607). Supplemental oxygen was

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added to the inspired air to maintain a normal arterial oxygen tension as determined on an Instrumentation Laboratory blood-gas analyzer (113-S1). The right femoral artery and vein were isolated and catheterized, with the catheters advanced to the aortic arch and inferior vena cava. Arterial pressure was monitored with a Statham pressure transducer (P23Db) and an Electronics for Medicine (Model Dr-8) oscillograph. A left thoracotomy was performed through the fifth intercostal space, the pericardium incised, and the heart exposed. Gauze, damped in saline (0.9%), was placed on the left ventricular epicardium to prevent the epicardial surface from drying. Heparin (500 U/kg) was administered and a blood sample was taken for determination of arterial PO_2, PCO_2, and pH. Blood samples were analyzed immediately, and if required, ventilation was adjusted to maintain blood gases within normal ranges and sodium bicarbonate was given to maintain arterial pH above 7.30.

In eight animals 1-[7,8-^{3}H]norepinephrine (Amersham, 30–40 Ci/mmole) was administered (2.5 μCi/kg, iv) as described by Kaye and Tyce (8). The protocol involved the infusion of the [^{3}H]norepinephrine over a 5-min period, followed by a 20-min incubation period, which enabled the labeled norepinephrine to become incorporated into the neuronal pools and to clear the extracellular space. At the end of the 20 min the heart was rapidly excised and placed in chilled saline (0°). In five additional animals, cocaine was administered (iv) to block neuronal uptake, 330 μg/kg/min for 15 min prior to the [^{3}H]norepinephrine infusion and 66 μg/kg/min during the infusion and incubation periods (9).

Using a grid system, the left ventricle was divided into 33 sections in a cold room (25 transmural free wall sections and 8 septal sections) (Fig. 1). By utilizing the papillary muscles as landmarks it was possible to section the free wall into five "rows" from base to apex and five "columns" from anterior to posterior. All tissue samples were minced, weighed, and placed into tubes containing 0.4 *N* perchloric acid (0°). The minced sample was homogenized with two 10-sec bursts of a polytron. The homogenate was centrifuged at 10,000*g* for 20 min, the supernatant was decanted and frozen for later analysis. Two milliliters of the supernatant was placed into a tube containing 150 mg of alumina, which had been prepared according to the method of Anton and Sayre (10), and 3 ml of 2 *M* Tris–0.5 *M* EDTA buffer, pH 8.6 (at this pH norepinephrine is bound to the alumina, whereas the *o*-methylated metabolites are not). This solution was vortexed thoroughly, and the supernatant was aspirated. The alumina was washed three times with distilled water (2–3 ml) and the norepinephrine was eluted from the alumina by addition of 1 ml of 0.05 *N* perchloric acid and thorough vortexing. The acid was pipetted into a scintillation tube containing 10 ml of a toluene-based cocktail (3a20, Research Products International) and 3 ml of a detergent (Triton X-100 scintillation grade, Research Products International). Samples were counted for 10 min in a Packard Tri-Carb liquid scintillation spectrometer. Quenching standards were used to calculate efficiency which averaged 44%. [^{3}H]Norepinephrine standards were used to calculate the percentage yield of the alumina extraction procedures, which averaged 41%. All sample counts were corrected for tissue weight, efficiency, percentage yield, and counting time, yielding results expressed as disintegrations per minute per gram (dpm/g). When duplicate [^{3}H]norepinephrine uptake values were compared, the coefficient of variation (r^2) was 0.95.

Differences among the means of the 33 sections for each of the two groups (control and cocaine-treated) were analyzed by analysis of variance. If the *F* value was significant ($P < 0.05$), a further statistical analysis was performed on the 25 free wall sections as follows: the data for individual sections were combined into 5 horizontal "rows" for making comparisons between the base and apex and parallel intermediate regions and into 5 vertical "columns" for making comparisons between the anterior and posterior regions and parallel intermediate regions, using analysis of variance and multiple comparison testing.

Results. The results obtained in the eight control animals are shown in Figs. 1–3. Shown in Fig. 1 are the [³H]norepinephrine uptake data for all 33 tissue sections. Analysis of variance of the 33 sections was significant ($P < 0.05$). Since this indicated that the uptake of [³H]norepinephrine was nonuniform, further statistical testing was performed. Shown in Fig. 2 are the [³H]norepinephrine uptake data for the larger regions of the left ventricular free wall obtained by combining sections into "rows." Analysis of variance was significant ($P < 0.05$), indicating that [³H]norepinephrine uptake was nonuniform in a base to apex direction. Further analysis revealed that uptake was significantly greater in the two most basilar "rows" than in the apical "row." Uptake in the two intermediate "rows" adjacent to the apex was not significantly different from that in the apical "row" or the two most basilar "rows."

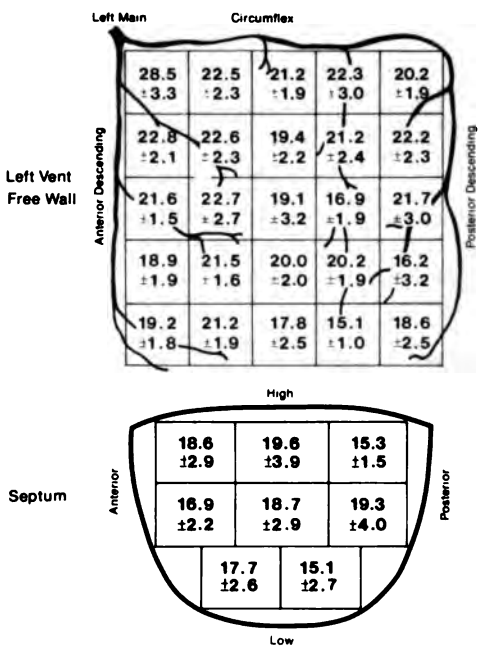

FIG. 1. [³H]Norepinephrine uptake in 25 regions of the left ventricular free wall and 8 regions of the interventricular septum obtained by systematically sectioning the ventricle into 33 tissue samples. The values shown, multiplied by 10^3, represent disintegrations per minute per gram. They are the means and standard errors for eight control animals.

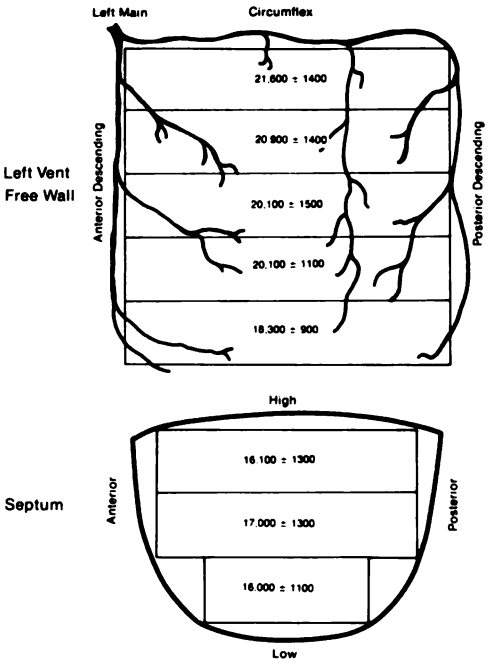

FIG. 2. Myocardial [³H]norepinephrine uptake data (dpm/g) as analyzed in a base to apex direction. Values are mean ± SEM.

Shown in Fig. 3 are the [³H]norepinephrine uptake data for the larger regions of the left ventricular free wall obtained by combining sections into "columns." Analysis of variance was insignificant ($P > 0.1$), indicating that [³H]norepinephrine uptake was uniform in an anterior to posterior direction. Analysis of variance of the eight sections of the septum was insignificant ($P > 0.05$), indicating that [³H]norepinephrine uptake was uniform in the septum.

The average [³H]norepinephrine uptake value for the entire left ventricular free wall was 20,000 ± 1200 dpm/g, whereas that for the septum was 16,000 ± 2000 dpm/g. The difference was not statistically significant ($P > 0.1$).

Results obtained in the cocaine-treated animals are shown in Fig. 4. Depicted are the [³H]norepinephrine uptake data for all 33 tissue sections. Analysis of variance of the 33 sections was insignificant ($P > 0.1$), indicating that nonneuronal [³H]norepinephrine uptake in the left ventricular free wall and septum was uniform. The average

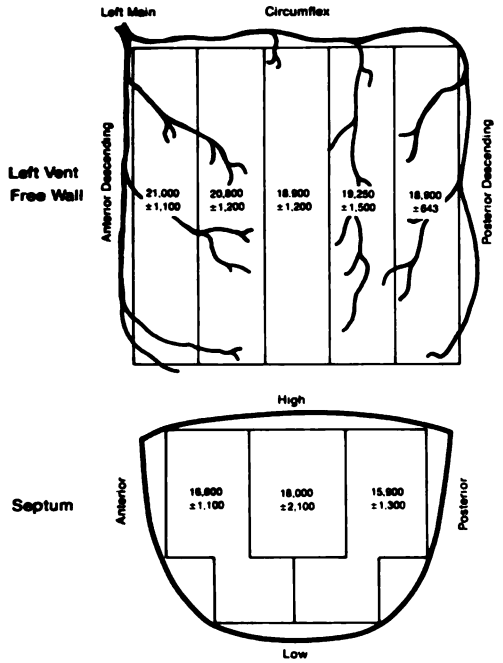

FIG. 3. Myocardial [³H]norepinephrine uptake data (dpm/g) as analyzed in an anterior to posterior ventricular wall direction. Values are mean ± SEM.

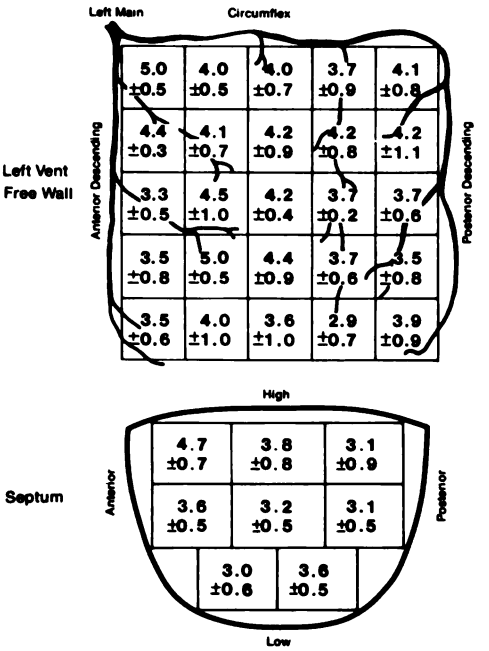

FIG. 4. [³H]Norepinephrine uptake portrayed as in Fig. 1, but for five cocaine-treated animals.

[³H]norepinephrine uptake value for the entire left ventricular free wall was 4000 ± 500 dpm/g, whereas that for the septum was 3500 ± 500 dpm/g. [³H]Norepinephrine uptake for the free wall and septum in the cocaine-treated animals was significantly less than that in control animals, with the values averaging approximately 20% of those obtained in the control animals.

Discussion. The major findings of this study are: (i) that a regional difference in neuronal [³H]norepinephrine uptake was observed between the base and apex of the ventricular free wall, with the uptake being approximately 20% greater in the base than in the apex, and (ii) nonneuronal [³H]norepinephrine uptake is uniform throughout the left ventricle, and it amounts to approximately 20% of that taken up by the normal ventricle.

The method of [³H]norepinephrine uptake to assess cardiac sympathetic efferent innervation has been extensively utilized by several laboratories (8, 11, 16). [³H]Norepinephrine uptake was reported to be a more reliable indicator of sympathetic innervation than norepinephrine content of the heart (8). Alumina extraction of norepinephrine eliminates methylated metabolites, which have the tritium label, but not the deaminated metabolites. However, in the heart the deaminated metabolites are reported to constitute only 1–2% of the recovered label after tracer norepinephrine uptake studies (17). The recovery of norepinephrine in this study was 41%, which is lower than that usually reported in the literature. However, the recovery was consistent among the experiments and the coefficient of variation of the procedure was 0.95. When the absolute uptake values were normalized for the dose of tracer norepinephrine, and compared to those of Kaye and Tyce (8), also normalized for the dose of tracer norepinephrine, the values were virtually identical. Thus, we believe our results reflect the uptake of [³H]norepinephrine and the pattern of sympathetic innervation.

In the canine left ventricle, the sympathetic innervation has been termed

"patchy" (7) and two earlier studies suggested that innervation is greater in the base than in the apex (6, 7). Evidence that the catecholamine content of the base is higher than that of the apex has also been provided in a more recent study (8). However, in that study, no difference of [^{3}H]-norepinephrine uptake was found between the base and apex. The reason for the difference in findings between that study and the present study is not clear. One possible explanation is a more discrete separation of the ventricular free wall into apical and basilar regions in the present study. Only the [^{3}H]norepinephrine uptake value for the apical "row" was significantly different from that for the most basilar "rows," whereas the value for the intermediate "row" immediately adjacent to the apical "row" was not significantly different from the two most basilar "rows." Others have demonstrated that the sympathetic efferent innervation courses in the epicardium from the anterior basilar region to the apical and posterior regions (3). In addition, it has been shown that when the canine heart is surgically denervated, reinnervation returns in a base to apex sequence (11). The present results support the notion of a greater sympathetic innervation in the base than in the apex. The [^{3}H]norepinephrine uptake was approximately 20% greater in the basilar region than in the apical region.

One reason for regional variations in sympathetic innervation of the ventricle could be an association of the sympathetic nerves with the coronary vasculature. It has been shown that sympathetic nerves travel in the adventitia of the coronary conductance vessels before innervation of the myocardium (12, 13). We have shown in another study (14) that by applying phenol to selective sites on the myocardium and epicardial conductance vessels it is possible to produce a regional sympathectomy of the left ventricle.

In cocaine-treated animals the [^{3}H]norepinephrine uptake was uniform throughout the ventricle. These results favor the conclusion that the greater basilar [^{3}H]-norepinephrine uptake in the normal animal was due to a regional difference in sympathetic innervation rather than to a regional difference in myocardial blood flow or some other experimental variable. More direct evidence against a regional difference in blood flow has also been obtained in other studies on this animal preparation, using the microsphere method to measure blood flow. The nonneuronal uptake sites consist of myocytes, vascular smooth muscle, connective tissue and fibroblasts (15), which are distributed homogeneously in the myocardium.

The physiological significance of a nonuniform distribution of sympathetic nerves between the base and apex of the ventricular wall is not elucidated by the present study. Others have demonstrated localized changes in myocardial function by stimulating discrete branches of the cardiac sympathetic nerve supply (2) which suggests that overall ventricular function may be modulated by regional differences in cardiac sympathetic nerve function.

In conclusion, the present study provides further evidence of a nonuniform distribution of sympathetic neurons between the base and apex of the canine left ventricle, with a greater density of neurons in the base than in the apex. The regional distribution of sympathetic neurons otherwise appears to be uniform in the left ventricular free wall and septum. The study also indicates that the nonneuronal uptake of norepinephrine is uniform in the left ventricle, and the nonneuronal uptake constitutes approximately 20% of labeled norepinephrine uptake.

1. Martins JB, Zipes DP. Effects of sympathetic and vagal nerves on recovery properties of the endocardium and epicardium of the canine left ventricle. Circ Res 46:100–110, 1980.
2. Randall W. Neural Control of the Heart. New York, Oxford Univ Press, 1977.
3. Kaye MP, Brynjolfsson GG, Geis WP. Chemical epicardiectomy. Cardiologia 53:139–149, 1968.
4. Martins JB, Zipes DP. Epicardial phenol interrupts refractory period responses to sympathetic but not vagal stimulation in canine left ventricular epicardium and endocardium. Circ Res 47:33–40, 1980.
5. Randall WC, Szentivanyi M, Pace JB, Wechsler

JS, Kaye MP. Patterns of sympathetic nerve projections onto the canine heart. Circ Res 22:315–323, 1968.
6. Angelakos ET. Regional distribution of catecholamines in the dog heart. Circ Res 16:39–44, 1965.
7. Dahlstrom A, Fuxe K, Mya-Tu M, Zetterstrom BEM. Observations on adrenergic innervation of dog heart. Amer J Physiol 209:689–692, 1965.
8. Kaye MP, Tyce GM. Norepinephrine uptake as an indicator of cardiac reinnervation in dogs. Amer J Physiol: Heart Circ Physiol 4:H289–H294, 1978.
9. Levy MN, Blattberg B. The influence of cocaine and desipramine on the cardiac responses to exogenous and endogenous norepinephrine. Eur J Pharmacol 48:37–49, 1979.
10. Anton AH, Sayer DF. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 138:360–375, 1962.
11. Kaye MP, Randall WC, Geis WP, Priola DV. Chronology and mode of reinnervation of the surgically denervated canine heart: Functional and chemical correlates. Amer J Physiol: Heart Circ Physiol 2:H431–H437, 1977.
12. Denn MJ, Stone HL. Autonomic innervation of dog coronary arteries. J Appl Physiol 41:30–35, 1975.
13. McKibben JS, Getty R. A comparative morphologic study of the cardiac innervation in domestic animals. I. The canine. Amer J Anat 122:533–544, 1968.
14. Chilian WM, Boatwright RB, Shoji T, Griggs Jr DM. Evidence against significant resting sympathetic coronary vasoconstrictor tone in the conscious dog. Circ Res 49:866–875, 1981.
15. Trendelenburg U. The extraneuronal uptake and metabolism of catecholamines in the heart. In: Paton DM, ed. The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines. New York, Raven, 1976.
16. Coglianese C, Randall WC, Filkins JP. The relative density of sympathetic nerve terminals in the canine right atrium. Proc Soc Exp Biol Med 154:127–130, 1977.
17. Kaye MP, Wills DJ, Tyce GM. Nerve growth factor-enhanced reinnervation of surgically denervated canine heart. Amer J Physiol: Heart Circ Physiol 5:H624–H628, 1979.
18. Herting G, Axelrod J, Whitby LG. Effect of drugs on the uptake and metabolism of ^{3}H-norepinephrine. J Pharmacol Exp Ther 134:146–153, 1961.

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Stimulation of Mammary Tumorigenesis and Suppression of Uterine Adenomyosis by Temporary Inhibition of Pituitary Prolactin Secretion during Youth in Mice[1] (41492)

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Abstract. The effects of daily subcutaneous injections of 0.2 mg CB-154 (bromocriptine-mesilate), a potent suppressor of pituitary prolactin secretion, between 4 and 11 weeks of age on the occurrence of spontaneous mammary tumors and adenomyosis, a hyperplasia of endometrial tissue, were studied in the SHN strain of virgin mice. While there was little difference in mammary tumor incidence between experimental and control mice until 9 months of age, mammary tumor incidence in the experimental mice given CB-154 was significantly enhanced and surpassed that in the control after 10 months; 25 (53.2%), 31 (66.0%), and 36 (75.5%) of 47 experimental mice and 20 (27.5%), 23 (33.3%), and 34 (49.3%) of 69 control mice developed mammary tumors at 10, 11, and 12 months of age, respectively. In contrast, no adenomyosis appeared in 39 experimental mice at necropsy at 12 months of age, while 15 (46.9%) of 32 control mice developed it. Furthermore, five mice (15.6%) of the control had numerous subserosal nodules, an advanced state of adenomyosis. No significant correlation was observed in the control mice between the occurrence of mammary tumors and that of adenomyosis.

Mammary gland DNA synthesis, which is primarily controlled, in part, by prolactin (1, 2), is a limiting factor for mammary tumorigenesis (3, 4). DMBA (7,12-dimethylbenz[*a*]anthracene)-induced mammary tumorigenesis was much more marked in rats given DMBA at proestrus, when both circulating prolactin and mammary gland DNA synthesis were high, than in rats given DMBA at diestrus, when prolactin and DNA synthesis were low (5). Suppression by CB-154 of the high prolactin at proestrus resulted in the decline of mammary gland DNA synthesis and the inhibition of DMBA-induced mammary tumorigenesis. By contrast, the single prolactin injection elevated mammary gland DNA synthesis and stimulated DMBA-induced mammary tumorigenesis (5). Moreover, temporary suppression of pituitary prolactin secretion during youth protected markedly spontaneous mammary tumor development in rats (6, 7). Mammary gland DNA synthesis in this species is high only during youth with a peak around 7 weeks of age decreasing thereafter with increasing age (5, 8). Russo and Russo (9) also found that mammary gland of 50-day-old virgin rats contained much higher number and labeling index of terminal ducts and end buds, from which DMBA-induced mammary tumors arise, than the glands of 180-day-old virgin and multiparous rats. Thus, prolactin suppression after the peak of mammary gland DNA synthesis (11–18 weeks of age) had little effect on prophylaxis of mammary tumors at advanced ages (6, 7).

On the other hand, mammary gland DNA synthesis in SHN mice, a high mammary tumor strain, changes little with age and much higher than that in rats (10). Although chronic prolactin suppression was found to inhibit markedly spontaneous mammary tumor development in C3H mice (11–13), no data are available in this species on the effects of temporary suppression of prolactin secretion during youth on mammary tumorigenesis. The effects of short-term

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and long-term suppression of prolactin secretion on mammary tumors are two different problems.

It has recently been observed that SHN virgin mice develop spontaneously adenomyosis, hyperplasia of endometrial tissue, both glandular and stromal components in the myometrium (14, 15). The development of adenomyosis is strongly prolactin dependent in the presence of ovarian hormones; ectopic pituitary grafts have been reported to enhance the appearance of the condition in intact animals (14, 15), however, the mechanism of its development is little understood.

The primary objective of this experiment was to study the effects of temporary suppression of prolactin secretion during youth on the development of spontaneous mammary tumors and adenomyosis at advanced ages in SHN mice.

Materials and Methods. *Mice.* SHN strain of mice maintained by brother × sister mating were used at the 45th generation. One of the characteristics of this strain is the high and early development of mammary tumors; the final incidence and onset age of tumors in virgin mice are 100% and 8.9 months, respectively (16). They also develop adenomyosis after 7 months of age with the incidence of about 50% at 12 months (14, 15). Throughout the experiment, five or six mice each were kept in Teflon cages (15 × 18 × 13 cm) with wood shavings, maintained in an air-conditioned (24 ± 0.5°C and 65–70% relative humidity) and artificially illuminated (14 hr of light from 5:00 AM to 7:00 PM) animal room and provided with a commercial diet and tap water *ad libitum*.

CB-154 treatment. A daily dose of 0.2 mg CB-154 (bromocriptine-mesilate: Sandoz Ltd., Basel, Switzerland), a potent suppressor of pituitary prolactin release (17–19), suspended in 0.05 ml olive oil with the Teflon glass homogenizer was injected subcutaneously each into 47 experimental mice between 4 and 11 weeks of age. Sixty-nine control mice received vehicle only.

Mammary tumorigenesis. Each mouse was checked for palpable mammary tumors every 7 days between 3 and 12 months of age.

Adenomyosis. All mice were killed by cervical dislocation at 12 months of age. Uteri were fixed in Bouin's solution, embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin–eosin for histological determination of adenomyosis (14, 15).

Statistics. Statistical evaluation of mammary tumorigenesis was performed using the multiple classification method (two-way analysis of variance) (20). By this method, the statistical sequence of the incidence and onset age of mammary tumors could be determined simultaneously. The difference in the incidence of adenomyosis or subserosal nodules was evaluated by χ^2 test.

Results. The results of mammary tumor development are presented in Fig. 1. In both experimental and control groups, the cumulative incidence of mammary tumors increased with age. However, the increasing rate was more marked in the experimental mice given CB-154 than in the control, especially after 10 months. The number (and percentage) of mice with tumors at 10, 11, and 12 months were 25 (53.2%), 31 (66.0%), and 36 (75.5%) of 47 experimental mice and 20 (27.5%), 23 (33.3%), and 34 (49.3%) of 69 control mice, respectively. Therefore, mammary tumorigenesis in the experimental group was significantly higher than that in the control

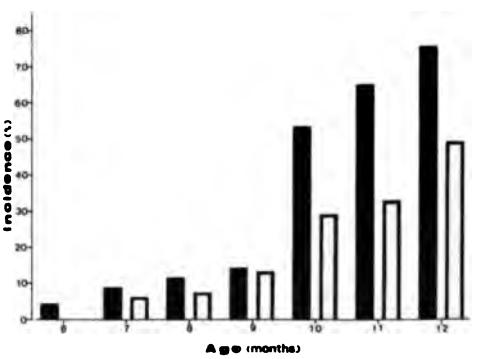

FIG. 1. Cumulative incidence of mammary tumors in experimental mice receiving CB-154 between 4 and 11 weeks of age (■) and the controls given vehicle only (□).

when evaluated by analysis of variance ($P < 0.05$).

In contrast, none of 39 experimental mice developed adenomyosis by 12 months of age, whereas in the controls, 15 or 46.9% of 32 mice developed it and five mice (15.6%) further had several subserosal nodules. The differences between experimental and control groups in the incidences of these pathological endometrial states were statistically highly significant ($P < 0.01$) (Table I).

No significant relationship was seen in the control mice between the occurrence of mammary tumors and that of adenomyosis; 6 out of 10 mice bearing mammary tumors (60%) and 9 out of 22 mice with no tumors (41%) developed adenomyosis.

The numbers of mice which died without tumors during the experiment were two and three in the experimental and control groups, respectively.

The body weight at the beginning of CB-154 injection (4 weeks of age) was 17.6 ± 0.2 (SE) g (pooled data of experimental and control groups) and the weights at the end of injection (11 weeks of age) were 26.9 ± 0.5 and 27.7 ± 0.4 g in the experimental and control groups, respectively, showing no difference between groups.

Discussion. This study shows that mice treated with CB-154 at an early age enhanced mammary tumorigenesis when compared with the control. Two major factors could be responsible for the enhanced mammary tumorigenesis seen in this study—pituitary and ovarian secretion of mammotropic hormones and mammary gland susceptibility to these hormones. Pituitary prolactin and ovarian estrogen and progesterone are prerequisite for the development of mammary tumors in mice, while established mammary tumors in many strains do not require hormonal support and, therefore, are autonomous (21). The enhancement of mammotropic hormone secretion as a result of terminating CB-154 treatment is unlikely, since normal reproductivity was observed in studies involving chronic administration of CB-154 (7, 22) and prolactin secretion at an advanced age was not altered by the treatment (7).

Mammary gland susceptibility to mammotropic hormones may be more important for normal and neoplastic mammary gland development than the secretion of mammotropic hormones (2). It has been reported that mammary glands exposed to abnormal hormonal conditions during early development show an increased susceptibility to mammotropic hormones (2). The enhanced mammary tumorigenesis in this study may be ascribed to the long-term effects of stimulated mammary gland susceptibility.

In any case, the present results suggest that the temporary inhibition of mammary gland DNA synthesis through prolactin suppression (6, 7) is not effective in preventing mammary tumorigenesis in species where mammary gland DNA synthesis continues at a high rate throughout the lifetimes.

The occurrence of adenomyosis at an advanced age (12 months) was completely eliminated by the temporary CB-154 treatment. While no information is available on the mechanism of adenomyosis development in mice except for its high prolactin dependency (14, 15), this study has demonstrated that there is a critical period for its

TABLE I. INCIDENCE OF ADENOMYOSIS AT 12 MONTHS OF AGE IN EXPERIMENTAL AND CONTROL MICE

Group[a]	No. of mice	No. (and %) of mice with Adenomyosis	No. (and %) of mice with Subserosal nodules
Experimental	39	0 (0)[b]	0 (0)[d]
Control	32	15 (46.9)[c]	5 (15.6)[e]

[a] Experimental and control mice received daily subcutaneous injections of CB-154 (0.2 mg) and vehicle only for 7 weeks between 4 and 11 weeks of age, respectively.
b/c; d/e: $P < 0.01$.

expression similar to that observed in rats for spontaneous mammary tumors (6, 7). Normal uterine growth is dependent upon estrogen and progesterone from the ovary and the susceptibility of uterine cells to these hormones changes little with age (23). However, exposure of uterus to abnormal hormonal conditions during the early developmental stages often induces a decrease in the susceptibility to estrogen (24). Thus, the present findings may indicate a continuous decline in uterine susceptibility to prolactin and ovarian steroid hormones as a result of CB-154 injection during youth and this could contribute to the complete elimination of adenomyosis at advanced ages.

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1. Nagasawa H. Prolactin: Its role in the development of mammary tumours. Med Hypoth 5:1117–1121, 1979.
2. Nagasawa H. Hormones and experimental mammary tumorigenesis. In: Nagasawa H and Abe K, eds. Hormone Related Tumors. Tokyo/Berlin, Japan Sci Soc Press/Springer-Verlag, pp137–163, 1981.
3. Nagasawa H. Mammary gland DNA synthesis as a limiting factor for mammary tumorigenesis: Forum. IRCS Med Sci 5:405–408, 1977.
4. Nagasawa H. Causes of age-dependency of mammary tumour induction by carcinogens in rats. Biomedicine 34:9–11, 1981.
5. Nagasawa H, Yanai R, Taniguchi H. Importance of mammary gland DNA synthesis on carcinogen-induced mammary tumorigenesis in rats. Cancer Res 36:2223–2226, 1976.
6. Nagasawa H, Morii S. Prophylaxis of spontaneous mammary tumorigenesis by temporal inhibition of prolactin secretion in rats at young ages. Cancer Res 41:1935–1937, 1981.
7. Nagasawa H, Morii S. Inhibition by early treatment with CB-154 (bromocriptine) of spontaneous mammary tumor development in rats with no side-effects. Acta Endocrinol 101:51–55, 1982.
8. Nagasawa H, Yanai R. Frequency of mammary cell division in relation to age: Its significance in the induction of mammary tumors by carcinogen in rats. J Nat Cancer Inst 52:609–610, 1974.
9. Russo J, Russo IH. DNA labeling index and structure of rat mammary gland as determinants of its susceptibility to carcinogenesis. J Nat Cancer Inst 61:1451–1459, 1978.
10. Nagasawa H. The cause of species differences in mammary tumorigenesis: Significance of mammary gland DNA synthesis. Med Hypoth 5:499–510, 1979.
11. Yanai R, Nagasawa H. Inhibition of mammary tumorigenesis by ergot alkaloids and promotion of mammary tumorigenesis by pituitary isografts in adreno-ovariectomized mice. J Nat Cancer Inst 48:715–719, 1972.
12. Welsch CW, Gribler C. Prophylaxis of spontaneous mammary carcinoma in C3H/HeJ female mice by suppression of prolactin. Cancer Res 33:2939–2946, 1973.
13. Welsch CW, Gribler C, Clemens JA. 6-methyl-8-β-ergolineacetonitrile (MEA)-induced suppression of mammary tumorigenesis in C3H/HeJ female mice. Eur J Cancer 10:595–600, 1974.
14. Mori T, Nagasawa H, Takahashi S. The induction of adenomyosis in mice by intrauterine pituitary isografts. Life Sci 29:1277–1282, 1981.
15. Mori T, Nagasawa H, Nakajima H. Strain-difference in the induction of adenomyosis by intrauterine pituitary grafting in mice. Lab Anim Sci 32:40–41, 1982.
16. Nagasawa H, Yanai R, Taniguchi H, Tokuzen R, Nakahara W. Two-way selection of a stock of Swiss albino mice for mammary tumorigenesis: Establishment of two new strains (SHN and SLN). J Nat Cancer Inst 57:425–430, 1976.
17. Floss HG, Cassady JM, Robbers JE. Influence of ergot alkaloids on pituitary prolactin and prolactin-dependent process. J Pharmacol Sci 62:699–715, 1973.
18. Barfknecht CF, Rusterholz DB, Parsons JA. Inhibition of prolactin by ergoline congeners. J Med Chem 17:308–312, 1974.
19. Flückiger EW. Lactation inhibition by ergot drugs. In: Yokoyama A, Mizuno H, Nagasawa H, eds. Physiology of Mammary Glands. Tokyo/Baltimore, Japan Sci Soc Press/Univ Park Press, pp71–82, 1978.
20. Snedecor CW. Statistical Methods, 5th ed. Ames, Iowa State Univ Press, pp241–253, 1966.
21. Welsch CW, Nagasawa H. Prolactin and murine mammary tumorigenesis: A review. Cancer Res 37:951–963, 1977.
22. Welsch CW, Morford LK. Influence of chronic treatment with 2-bromo-α-ergocriptine (CB-154) on the reproductive and lactational performance of the C3H/HeJ female mouse. Experientia 30:1353–1355, 1974.
23. Finn CA, Martin L. The cellular response of the uterus of the aged mouse to oestrogen and progesterone. J Reprod Fertil 20:545–547, 1969.
24. Mori T. Effects of postpubertal oestrogen injections on mitotic activity of vaginal and uterine epithelial cells in mice treated neonatally with oestrogen. J Endocrinol 64:133–140, 1975.

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Blood-Borne Vasoconstrictor Stimulates Release of [^{14}C]Arachidonate from Prelabeled Isolated Perfused Rabbit Gastrocnemius Muscle[1] (41493)

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Abstract. The effect of a blood-borne vasoconstrictor on vascular resistance and arachidonate release was examined using isolated, perfused rabbit gastrocnemius muscle. Replacement of blood by perfusion with Tyrode's solution caused a marked drop in vascular resistance. Addition of rabbit plasma or the plasma factor partially purified from human blood restored vascular resistance in a concentration dependent manner. The pressor effect of the plasma factor was unaffected by concentrations of phentolamine (2.5 $\mu g \cdot ml^{-1}$) and sar^1-ala^8-angiotensin II (75 $\mu g \cdot ml^{-1}$) sufficient to completely block the effects of norepinephrine and angiotensin II, respectively. However, the pressor effects of the factor were inhibited by indomethacin in a concentration-dependent manner. The factor produced a concentration dependent (r = 0.95, n = 12) increase in release of [^{14}C]arachidonate from prelabeled preparations. This release was blocked by indomethacin. These results are consistent with the hypothesis that the pressor effects of the factor result from its effects upon arachidonate metabolism.

Evidence indicates the existence of an unidentified humoral factor which is important in the maintenance of basal vascular resistance (1–4). Perfusion of the isolated dog gracilis muscle with a blood-free physiological salt solution causes vasodilation and a concomitant loss of the autoregulatory response to changes in perfusion pressure. Addition of blood plasma to the perfusate restores both the autoregulatory response and vascular resistance. Norepinephrine causes vasoconstriction but does not restore the autoregulatory response (1). Bohr and Sobieski obtained a substance from plasma which produces contraction in vascular smooth muscle strips isolated from a variety of sites (2). We have partially purified a substance from blood plasma which restores vascular resistance and the autoregulatory response in isolated rabbit hearts perfused with Tyrode's solution (3, 4).

The mechanism of action of this plasma pressor has not been completely delineated. Bohr and Johansson found the action of their plasma pressor to be unlike that of a variety of vasoactive agents (norepinephrine, angiotensin II, histamine, serotonin, and vasopressin) (5). We have found that the pressor effects of the plasma factor can be blocked with inhibitors of prostaglandin synthesis such as indomethacin (4). Additionally, the partially purified factor stimulates the conversion of arachidonate to prostaglandins and thromboxanes in isolated perfused rabbit hearts (3, 4), human platelet suspensions (6), and cell-free enzyme preparations (6–8). The factor also stimulates the conversion of arachidonate to 12-hydroperoxyeicosatetraenoic acid [12-HPETE] via the reaction catalyzed by lipoxidase (6). The factor alters the relative amounts of products formed from arachidonate, increasing formation of vasoconstrictors, such as prostaglandin $F_{2\alpha}$, with respect to vasodilators, such as prostaglandin I_2. This last effect could result from factor-stimulated synthesis of 12-HPETE

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which inhibits prostaglandin I_2 synthase (9). The increase in the ratio of vasoconstrictors to vasodilators might account for the pressor activity of the factor.

The factor may also increase the release of arachidonate from endogenous stores. In isolated rabbit hearts the factor increases prostaglandin E_2-like activity as assayed biologically with rat stomach fundus strips (3). Since exogenous arachidonate was not used in these experiments, prostaglandins must have been formed from endogenous arachidonate. The rate determining step in prostaglandin synthesis is the release of arachidonate from endogenous stores. Hence, the factor must have stimulated release of arachidonate.

To confirm this, we examined the effect of the factor on release of arachidonate in rabbit gastrocnemius muscle prelabeled with [^{14}C]arachidonate. Additionally, we compared the pressor effect of the factor with other vasoactive agents.

Methods. *Chemicals.* Stock solutions of all drugs and other vasoactive agents were prepared on the day of use. Norepinephrine (Levarterenol bitartrate, 0.2%, injection) was obtained from Winthrop; indigo carmine (0.8% injection) from Hynscott Wescott and Dunning; angiotensin II, histamine, serotonin, vasopressin, arachidonic acid and indomethacin from Sigma; phentolamine mesylate (injection) from Ciba; sar^1-ala^8-angiotensin II (saralasin) from Vega Biochemicals and rabbit serum albumin (Cohn fraction V) from ICN Pharmaceuticals. [^{14}C]Arachidonic acid (321 $Ci \cdot g^{-1}$) was purchased from New England Nuclear.

Indomethacin (100 mg) was dissolved in 2.0 ml 95% ethanol (37°) and diluted to 10 ml with modified Tyrode's solution (137 m*M* NaCl, 4 m*M* KCl, 1.8 m*M* $CaCl_2$, 0.5 m*M* $MgSO_4$, 0.35 m*M* Na_2HPO_4, 18 m*M* $NaHCO_3$, 5.6 m*M* glucose) containing 35 mg Na_2CO_3. Radiolabeled arachidonate was dissolved in the modified Tyrode's solution containing factor-free (4) albumin (2.5 $mg \cdot ml^{-1}$).

Plasma factor. The plasma factor was prepared as described previously (4) except that it was obtained from human blood instead of rabbit blood. The factor is normally bound to albumin in blood plasma but can be extracted from albumin or plasma with chloroform methanol (2:1) and purified with thin-layer chromatographic techniques. The extraction procedure yields 60 to 70% of the original pressor activity in plasma. The partially purified factor was dissolved in methanol and stored at −20°. Before use, the methanol was evaporated with nitrogen and the residue dissolved in modified Tyrode's solution containing factor-free albumin (2.5 $mg \cdot ml^{-1}$). For convenience, factor was reconstituted to the original volume from which it was extracted. Final factor concentrations are expressed as a percentage of the reconstituted stock solution.

Skeletal muscle and heart preparations. New Zealand male rabbits (2.2 to 3.2 kg) were anesthetized with sodium pentobarbital (40 $mg \cdot kg^{-1}$). The anesthetic was supplemented as required. A tracheotomy was performed and the lungs were mechanically ventilated during surgery. The skin of the right leg was removed and the gastrocnemius muscle exposed. All blood vessels except the major artery and vein which supply and drain the muscle were ligated. Heparin (1000 $unit \cdot kg^{-1}$) was administered before cannulation of the artery and vein. The excised muscle was placed in a humidified chamber and perfused with the modified Tyrode's solution (pH 7.4, 37°, saturated with 95% O_2, 5% CO_2).

Flow of the perfusate was maintained with the aid of a constant speed peristalic pump (Cole-Palmer). Pressure was measured with a pressure transducer (Statham P23Db) and recorded with a pen chart recorder (Cole-Palmer). Muscles were perfused for one hour to remove all residual blood before testing. In experiments using indomethacin, substances were tested in the following sequence: plasma, purified factor, indomethacin. Following the experiments the integrity of the vascular bed was examined by injecting indigo carmine solution (0.8%). Data from preparations in which partial blockage of the vascular bed was detected were discarded. Rabbit hearts were isolated and perfused as described

previously (3, 4). Briefly, the hearts were perfused through an aortic cannula with modified Tyrode's solution at 37°, pH 7.4, saturated with 95% O_2, 5% CO_2. Flow was adjusted to attain a basal pressure of 60 mm Hg. Aortic pressure was measured and recorded as in the experiments with the gastrocnemius muscle preparations.

In the experiments comparing the effect of the plasma factor to that of known vasoactive agents, the agonists were tested by bolus injection whereas the antagonists were infused at constant rate with the aid of a syringe pump (Harvard Instruments).

Radiolabeled prostaglandins and other products synthesized from [^{14}C]arachidonate were extracted from the venous effluent with ethyl acetate and identified by thin-layer chromatography as previously described (7). Identification was substantiated by comparing the migration rates of the free acids and their methylated derivatives to those of authentic standards using several solvent systems (7).

Results. *Pressor effects.* As perfusion with Tyrode's solution was started vascular resistance, which had dropped to a low value during cannulation of the artery, increased toward normal *in vivo* values. However, as the vascular system in the gastrocnemius muscle was purged of blood, vascular resistance decreased markedly, demonstrating that the net effect of switching from blood perfusion to perfusion with Tyrode's solution in this preparation is a decrease in vascular resistance (Fig. 1). When vascular resistance reached basal levels the perfusion pump speed was adjusted to produce a pressure of 30 mm Hg.

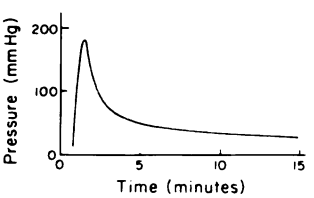

FIG. 1. Changes in perfusion pressure associated with the beginning of perfusion with Tyrode's solution in rabbit gastrocnemius muscle. Recorder tracing from one muscle preparation typical of all (15) preparations tested. Flow rate, 25 ml·min^{-1}·100 g^{-1}.

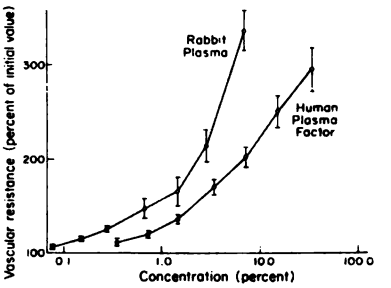

FIG. 2. Increases in vascular resistance produced by infusion of plasma and purified factor in isolated rabbit gastrocnemius muscle. Flow rate, 25 ± 4 ml·min^{-1}·100 g^{-1}, initial pressure = 30 mm Hg, resistance = pressure (mm Hg)·ml^{-1}·min·100 g, mean ± standard error. Data from six gastrocnemius muscle preparations.

The basal flow rate was 24.6 ± 3.9 ml·100 g^{-1}·min^{-1} (mean ± standard error, 15 gastrocnemius preparations). Infusion of rabbit plasma or partially purified human plasma factor caused a concentration dependent increase in vascular resistance (Fig. 2).

Comparison of factor with other vasoactive agents. Among the vasoactive agents tested (norepinephrine, angiotensin II histamine, serotonin, and vasopressin) only norepinephrine, angiotensin II, and histamine produced vasoconstriction in gastrocnemius muscle preparations (Fig. 3). Histamine produced vasoconstriction in high doses in gastrocnemius muscle but

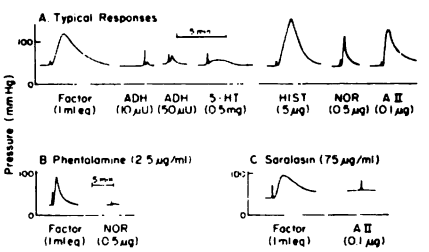

FIG. 3. Vascular effects of agonists and antagonists in isolated rabbit gastrocnemius muscle. Agonists were administered by bolus injection. Antagonists were infused at constant rates. Abbreviations: ADH, vasopressin; 5-HT, serotonin; HIST, histamine; NOR, norepinephrine; AII, angiotensin II. Recorder tracings from a single preparation are illustrative of the responses produced in all four muscle preparations tested. One milliliter equivalent (ml eq) is the amount extracted from one milliliter of blood plasma.

produced vasodilation in isolated rabbit hearts (Fig. 4). Serotonin also produced coronary vasodilation. The effects of norepinephrine and angiotensin II were completed blocked by phentolamine (2.5 $\mu g \cdot ml^{-1}$) and saralasin (75 $\mu g \cdot ml^{-1}$), respectively. Neither of these inhibitors had any effect upon factor-induced vasoconstriction (Fig. 3).

Effects on arachidonate metabolism. To study the release and metabolism of arachidonate the gastrocnemius muscle preparations were prelabeled with [^{14}C]arachidonate. Radiolabeled arachidonate (5 μCi in 1.0 ml) was injected into the perfusate in five aliquots at 2-min intervals. More than 90% of the label was retained by the preparation. Collection of samples was started 5 min after the last injection. Effluent (14 ml) was collected in a 4-min period. The radiolabeled substances identified in the venous effluent were arachidonate, prostaglandin E_2, and the stable derivative of prostaglandin I_2, 6-keto-prostaglandin $F_{1\alpha}$. Because of the low levels of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ recovered from the venous effluents using this prelabeling technique, effects of the factor on venous levels of these products could not be determined. Although both these products were detected in the venous effluent of all four of the muscle preparations tested, factor-induced statistically significant effects were not obtained. However, a correlation (r = 0.95, n = 12) between factor-induced vasoconstriction and release of [^{14}C]arachidonate was seen (Fig. 5, Table I). The release of arachidonate was not due to vasoconstriction per se because there was no correlation (r = 0.44) between the vasoconstriction produced by

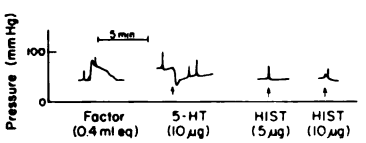

FIG. 4. Effects of factor, serotonin (5-HT), and histamine (HIST) on vascular resistance in isolated perfused rabbit hearts. Recorder tracings from one heart are typical of those seen in the five isolated hearts tested. Flow rate, 20 $ml \cdot min^{-1}$.

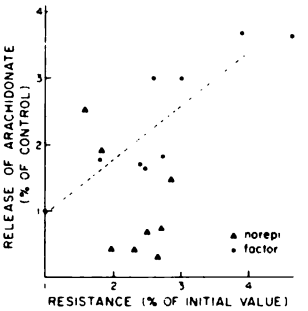

FIG. 5. Correlation between release of [^{14}C]arachidonate and factor-induced vasoconstriction. For factor: $y = 1.1X + 13$, $r = 0.95$ for norepinephrine: $y = 0.6X + 108$, $r = 0.44$. Data from four isolated gastrocnemius muscle preparations.

norepinephrine and the release of [^{14}C]-arachidonate (Fig. 5).

Indomethacin counteracts factor-induced vasoconstriction and prostaglandin synthesis in isolated rabbit hearts (3, 4). In gastrocnemius muscle preparations indomethacin had little effect on vascular resistance in the absence of factor, but did suppress factor-induced vasoconstriction in a concentration-dependent manner (Table II). Inhibition was proportional to the logarithm of indomethacin concentration ($y = 36.3x + 6.9$, $r = 0.97$, $n = 60$). The indomethacin concentration for 50% inhibition was 15 μM. A period of about 20 min was required for stabilization of vascular resistance after indomethacin infusion was started. After stabilization [^{14}C]arachidonate was administered by bolus injection 1 min before sample collection was started. Indomethacin reduced radiolabeled venous ^{14}C-prostaglandins both in the presence and absence of factor. It is of particular interest that indomethacin blocked the factor-induced increase in release of [^{14}C]arachidonate (Table I).

Discussion. Our results show that the factor which restores coronary vasoconstriction in isolated rabbit hearts (3, 4) also restores vasoconstriction in skeletal muscle. Our results confirm those of Bohr and Johansson (5) which show that the action of the plasma factor is unlike that of a variety of vasoactive agents.

The results reported herein substantiate

TABLE I. EFFECT OF VASOACTIVE FACTOR AND INDOMETHACIN ON RELEASE OF [^{14}C]ARACHIDONATE FROM LABELED RABBIT GASTROCNEMIUS MUSCLE

Factor concentration (%)	Indomethacin (mM)	Vasoconstriction (% of control)	Arachidonate (% of control)
0	0	100	100
15	0	212 ± 28	174 ± 9
35	0	285 ± 18	326 ± 23
35	0.2	132 ± 23	30 ± 5

Note. Repeated measurements were made on four muscle preparations, means ± standard error.

results we obtained using isolated rabbit hearts (3) which suggest that the factor stimulates release of arachidonate from endogenous stores. In this regard the relationship between indomethacin and factor is important. Indomethacin counteracts the effects of the factor on the cyclooxygenase reaction in a competitive manner (7). Indomethacin has been shown to block the release of arachidonate by phospholipases (10). Hence, indomethacin and the plasma factor have opposing effects on both the release of arachidonate and its conversion to endoperoxides. This finding is compatible with recent evidence for enzymatic coupling of phospholipase A_2 and cyclooxygenase activities (11).

The correlation between factor-induced arachidonate release and factor-induced vasoconstriction is consistent with the hypothesis that vasoaction of the factor results from its effects upon arachidonate metabolism. In addition to stimulating the release of arachidonate and its conversion to endoperoxide prostaglandins via the cyclooxygenase reaction (3, 4) the factor stimulates conversion of arachidonate to 12-HPETE by lipoxidase (6). This hydroperoxy fatty acid inhibits the formation of the vasodilator prostaglandin I_2 from endoperoxide prostaglandins (9). Lipoxidase activity has been found in vascular tissue (12). Hence the factor stimulates release of arachidonate but can simultaneously divert it from the formation of prostacyclin, a vasodilator, to vasoconstrictor prostaglandins (e.g., $PGF_{2\alpha}$).

Administration of exogenous arachidonate can produce vasodilation, presumably through formation of prostaglandin I_2 (13). However, we have shown that the plasma factor attenuates arachidonate-induced vasodilation while concurrently increasing the ratio of prostaglandin $F_{2\alpha}$ to prostaglandin I_2 which is formed (8). Arachidonate is transformed to prostaglandins, thromboxanes, hydroperoxy fatty acids, and leukotrienes (14). Both vasodilators and vasoconstrictors can be formed simultaneously, depending upon the activity of the enzymes present. The plasma factor has a marked effect on the activity of some of these enzymes. Thus, it could act by maintaining a balance between the vasocon-

TABLE II. INDOMETHACIN INHIBITION OF FACTOR-INDUCED VASOCONSTRICTION

Indomethacin (μM)	Factor concentration (%)			
	3.5	7.0	15	35
	Percentage inhibition			
0	0	0	0	0
6	32 ± 2	42 ± 2	28 ± 3	37 ± 4
50	84 ± 14	75 ± 12	68 ± 11	65 ± 24
200	94 ± 13	98 ± 14	89 ± 11	83 ± 6

Note. Percentage inhibition was calculated by dividing the factor-induced increase in vascular resistance in the presence of indomethacin by the increase in the absence of indomethacin, subtracting the quotient from 1.0, and multiplying that difference by 100. Data from four gastrocnemius preparations (mean ± standard error).

strictors and vasodilators formed from arachidonate.

1. Stainsby WN. Autoregulation in skeletal muscle. Circ Res 14(Suppl I):139–147, 1964.
2. Bohr DF, Sobieski, J. A vasoactive factor in plasma. Fed Proc 27:1396–1398, 1968.
3. Moretti RL, Abraham S, Ecker RR. The stimulation of cardiac prostaglandin production by blood plasma and its relationship to the regulation of coronary flow in isolated isovolumic rabbit hearts. Circ Res 39:231–238, 1976.
4. Moretti RL, Abraham S. Stimulation of microsomal prostaglandin synthesis by a blood plasma constituent which augments autoregulation and maintenance of vascular tone in isolated rabbit hearts. Circ Res 42:317–323, 1977.
5. Bohr DF, Johansson B. Contraction of vascular smooth muscle in response to plasma: Comparison with response to known vasoactive agents. Circ Res 19:593–601, 1966.
6. Moretti RL, Lin CY. Coronary vasoconstrictor extracted from blood plasma stimulates platelet lipoxidase activity. Prostaglandins 19:99–108, 1980.
7. Moretti RL, Abraham S. Stimulation of microsomal prostaglandin synthesis by a vasoactive material isolated from blood plasma. Prostaglandins 15:603–622, 1978.
8. Moretti RL. Blood borne vasoconstrictor lowers ratio of prostaglandin I_2 to other products formed from arachidonate. Prostaglandins Med 6: 223–232, 1981.
9. Moncada S, Gryglewski RJ, Bunting S, Vane JR. A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (prostaglandin X) which prevents platelet aggregation. Prostaglandins 12:715–737, 1976.
10. Franson RC, Eisen D, Jesse R, Lanni C. Inhibition of highly purified mammalian phospholipases A_2 by non-steroidal anti-inflammatory agents. Biochem J 186:633–636, 1980.
11. Erman A, Azuri R, Raz A. Enzymatic coupling of phospholipase A_2 and prostaglandin synthetase activities in subcellular-fractions from renal rabbit medulla. Biochem J 201:635–640, 1982.
12. Greenwald JE, Bianchine JR, Wong LK. The production of the arachidonate metabolite HETE in vascular tissue. Nature (London) 281:588–589, 1979.
13. Dusting GJ, Moncada S, Vane JR. Vascular actions of arachidonic acid and its metabolites in perfused mesenteric and femoral beds of the dog. Eur J Pharmacol 49:65–72, 1978.
14. Samuelsson B, Borgeat P, Hammarstrom S, Murphy RC. Introduction of a nomenclature: Leukotrienes. Prostaglandins 17:785–787, 1979.

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Effects of Garlic Products on Lipid Metabolism in Cholesterol-Fed Rats[1] (41494)

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Abstract. The effect of garlic prepared in several forms on lipid metabolism was studied in male rats fed a diet containing 1% cholesterol. Garlic was supplemented at 2% of the diet as fresh garlic in forms of ethanol extracted garlic residue, ethanol extract of garlic, whole garlic, and autoclaved garlic. Diets were fed for 4 weeks from 6 weeks of age. The supplementation of garlic products except ethanol-extracted garlic residue reduced plasma and liver cholesterol levels. The reduction in the plasma cholesterol by feeding garlic products was in very low density lipoprotein and low-density lipoprotein cholesterol fractions. Animals fed diets supplemented with garlic decreased liver glucose-6-phosphate dehydrogenase and malic enzyme activities and also reduced the liver weight, inquinal adipose tissue weight, liver total lipids, and plasma triglycerides. The hypocholesterolemic activity of garlic was contained in the ethanol extract and stable when autoclaved at 120° for 1 hr.

The blood cholesterol level has been reported as an independent risk factor contributing to the development of coronary heart diseases (1–3). Recent studies have shown that essential oil from garlic or onion reduces the blood cholesterol level in humans (4, 5) and animals (6, 7). The essential oil of garlic or garlic extract prevented lipid accumulation in the aorta and showed preventive effects against pathogenic atherosclerosis in rabbits fed an atherogenic diet (6, 7). An oral administration of garlic to human subjects depressed platelet aggregation (5, 8) and the blood glucose concentration (9). Garlic is used as a flavoring agent world wide. Garlic also has been known to have medicinal properties in oriental countries. Lyophilized garlic powder added at 2% level to a diet containing 1% cholesterol decreased the cholesterol and lipid levels in the blood and liver by increasing fecal excretion of neutral and acidic sterols in rats (10).

Garlic can be processed into different products and used. The objective of this study was to determine the effects of selected garlic products on lipid metabolism in rats including the determination of each lipoprotein cholesterol fraction and hepatic lipogenic enzyme activities.

Materials and Methods. Male Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were individually housed in stainless-steel cages in a room maintained at 22°–24° with about 50% relative humidity. The room was lighted from 06:00 to 18:00 hr. The composition of a basal diet (control group) containing 1% cholesterol is given in Table I. Garlic bulbs were obtained from a commercial source, peeled off, washed and ground by a blender for 2 min with an addition of water. A portion of ground garlic was lyophilized and used as whole garlic. The moisture contents of the fresh garlic bulb and dried garlic powder were 69.1 and 1.1%, respectively. Another portion of ground garlic was refluxed with 50% aqueous ethanol (v/v) in a 60° water bath for 2 hr, filtered, and ethanol was evaporated with N gas flow. The resulting solution was concentrated by lyophilization and used as garlic extract (22.1% of whole fresh garlic). The filtered garlic particles (8.9% of whole fresh garlic) were lyophilized and used as garlic residue. A portion of ground garlic was autoclaved for 1 hr at 120°, lyophilized and used as autoclaved garlic.

There were five dietary treatments: (1)

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TABLE I. COMPOSITION OF THE BASAL DIET

Ingredient	g/100 g diet
Casein[a]	20.00
Dextrose[a]	25.00
Corn starch[a]	40.35
Corn oil[b]	5.00
Cellulose[c]	2.50
Mineral mix[d,e]	4.00
Vitamin mix[f,g]	2.00
DL-Methionine[a]	0.15
Cholesterol[h]	1.00

[a] ICN Pharmaceuticals, Inc., Cleveland, Ohio.
[b] Tocopherol stripped, ICN Pharmaceuticals, Inc.
[c] Brown and Co., Boston, Mass.
[d] Supplied by J. T. Baker Chemical Co., Phillipsburg, N.J.
[e] Provided per kilogram diet: $CaCO_3$, 7.5 g; $CaHPO_4 \cdot 2H_2O$, 8.2 g; $CuSO_4 \cdot 5H_2O$, 0.023 g; $Fe(C_6H_{11}O_7)_2$, 0.9 g; KIO_3, 0.01 g; K_2HPO_4, 13.0 g; $MgSO_4 \cdot 7H_2O$, 4.8 g; $MnCO_3$, 0.2 g; NaCl, 4.5 g; Zn CO_3, 0.052 g.
[f] Vitamins except tocopherol, retinyl palmitate and cholecalciferol supplied by General Biochemicals, Chagrin Falls, Ohio; tocopherol, retinyl palmitate, cholecalciferol supplied by Hoffman-LaRoche, Nutley, N.J.
[g] Provided per kilogram diet; thiamin·HCl, 10 mg; riboflavin, 10 mg; nicotinic acid, 40 mg; calcium pantothenate, 30 mg; folic acid, 3 mg; inositol, 25 mg; biotin, 0.2 mg; vitamin B-12, 0.02 mg; menadione, 2 mg; retinyl palmitate, 10,000 IU; cholecalciferol, 2000 IU; DL-α-tocopheryl acetate, 120 IU; choline chloride, 1500 mg.
[h] Eastman Kodak Co., Rochester, N.Y.

basal diet (control), (2) control plus garlic residue, (3) control plus garlic extract, (4) control plus whole garlic, (5) control plus autoclaved garlic. Garlic products were added to the diet equivalent to 2% of a diet as fresh garlic (w/w) at the expense of corn starch. Rats were fed a stock diet for the 2-week preexperimental period. At 6 weeks of age, experimental diets and water were provided *ad libitum* for the next 4 weeks. Each dietary treatment was randomly assigned to 10 individually caged rats. The body weight and food consumption were determined weekly.

Chemical assay. Blood samples were taken into heparinized Vacutainer (Beckton-Dickinson, Dickinson and Co., Rutherford, N.J.) tubes by cardiac puncture; the rats were in a weakly anesthetized state following phenobarbital administration (after fasting for 16 hr). Immediately after the blood sampling the liver was excised, washed in chilled saline solution, blotted and cooled in crushed ice. A part of liver was used for enzyme assay and the rest of the liver was kept at −35°C until analysis of lipid components. The inquinal fat pads in both sides were removed at around caput epididymis and weighed.

Plasma triglycerides were analyzed enzymatically using Tri-Es (Harleco, Division of American Hospital Supply Co., Gibson, N.J.). Plasma lipoprotein cholesterol was separated by ultracentrifugation (11) and plasma total and each lipoprotein cholesterol fraction was determined using Beckman Cholesterol Analyzer-2 and enzymatic cholesterol assay kit (Beckman Instruments, Inc., Fullerton, Calif.). Liver lipids were determined gravimetrically after extraction according to the method of Folch *et al.* (12) and liver cholesterol was analyzed by the method of Kim and Goldberg (13).

Enzyme assay. Approximately 1.0 g of liver was homogenized in 9 ml of 0.15 *M* KCl and centrifuged at 20,000*g* for 1 hr. The resulting supernatant was used for determining enzyme activity and protein. Glucose-6-phosphate dehydrogenase (G-6-PDH) (EC 1.1.1.49) was assayed by the method of Kornberg and Horecker (14) and malic enzyme (ME) (EC 1.1.1.40) was assayed by the method of Ochoa (15). The reaction was initiated by adding the enzyme source to the medium. The assay was conducted at 30° by observing changes in absorbancy at 340 nm with a Beckman Model-25 Spectrophotometer (Beckman Instruments, Inc.). The protein content of the supernatant fraction was determined by the method of Lowry *et al.* (16). Enzyme activity was expressed as units per milligram protein where a unit was the amount of enzyme which converted one nanomole of substrate per minute at 30°.

Statistical analysis. The data were analyzed statistically using the analysis of variance technique and the least significant difference procedure was used in comparisons of treatment means (17).

Results. Table II and Fig. 1 summarize the results of the experiment. Rats fed the diet supplemented with garlic extract consumed less food than the control and whole

TABLE II. EFFECTS OF GARLIC PRODUCTS ON THE WEIGHT GAIN, FOOD CONSUMPTION, AND PLASMA AND LIVER COMPONENTS IN MALE RATS[a]

	Dietary treatments				
	Control	Garlic residue	Garlic extract	Whole garlic	Autoclaved garlic[b]
Weight gain, g/day	7.5 ± 0.5 A	7.2 ± 0.5 A,B	6.7 ± 0.3 B	6.4 ± 0.3 B	6.6 ± 0.4 B
Food intake, g/day	18.2 ± 1.4 A	17.1 ± 2.0 A,B	16.2 ± 1.4 B	17.2 ± 2.2 B	17.5 ± 1.2 B
Plasma triglycerides, mg/dl	133.4 ± 20.6 A	122.1 ± 16.7 A	91.4 ± 12.9 B	96.4 ± 13.0 B	97.3 ± 18.1 B
Plasma total cholesterol, mg/dl	102.9 ± 11.8 A	97.7 ± 17.6 A	74.6 ± 8.3 B	77.6 ± 7.8 B	78.3 ± 14.6 B
Plasma free cholesterol, mg/dl	29.1 ± 4.2 A	27.5 ± 3.1 A,B	24.6 ± 2.0 B	24.2 ± 2.6 B	24.0 ± 3.2 B
Liver weight[c]	5.07 ± 0.68 A	4.56 ± 0.45 A,B	3.93 ± 0.54 B	3.96 ± 0.35 B	4.11 ± 0.48 B
Inquinal fat pad weight[d]	1.11 ± 0.28 A	1.06 ± 0.16 A,B	0.85 ± 0.16 B	0.87 ± 0.20 B	0.89 ± 0.14 B
Liver total lipid, mg/g liver	72.1 ± 11.5 A	69.4 ± 13.8 A	41.4 ± 9.2 B	41.1 ± 7.0 B	52.6 ± 10.6 B
Liver total cholesterol, mg/g liver	15.88 ± 1.94 A	14.76 ± 2.47 A	6.33 ± 1.06 B,C	5.23 ± 0.84 C	8.83 ± 1.63 C
Liver free cholesterol, mg/g liver	4.88 ± 1.07 A	3.97 ± 0.80 A,B	2.33 ± 0.36 B	2.56 ± 0.29 B	3.38 ± 0.68 B
Liver G-6-PDH,[e] unit/mg protein	9.83 ± 1.36 A	9.20 ± 1.06 A	6.13 ± 1.34 B	6.97 ± 1.37 B	6.40 ± 1.74 B
Liver ME,[e] unit/mg protein	5.73 ± 1.03 A	5.82 ± 1.76 A	3.53 ± 0.82 B	3.69 ± 0.93 B	3.68 ± 0.68 B

[a] Mean ± standard deviation of 10 rats. Means bearing different letters at the same row are significantly different ($P < 0.05$).
[b] Mean ± standard deviation of 9 rats.
[c] Liver weight in g/100 g body weight.
[d] Two inquinal fat pad weights in g/100 g body weight.
[e] G-6-PDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme.

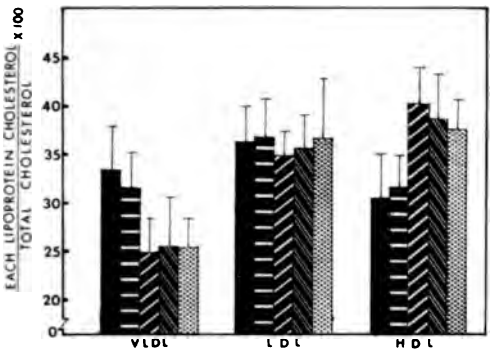

FIG. 1. Effects of garlic products on plasma very low density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol as a percentage of the plasma total cholesterol. ▬, Control; ▮▮▮, garlic residue; ▧, garlic extract; ▨, whole garlic; ▒, autoclaved garlic. Each bar indicates mean ± standard deviation of 10 rats except the autoclaved garlic group which is mean ± standard deviation of 9 rats.

garlic groups. Body weight gain of rats fed garlic products except garlic residue was decreased as compared with rats fed the control diet (Table II). The plasma concentrations of triglycerides, total cholesterol, and free cholesterol were decreased by the supplementation of garlic extract, whole garlic, or autoclaved garlic.

The plasma cholesterol was fractionated into each lipoprotein cholesterol and the percentage of each lipoprotein cholesterol to the plasma total cholesterol is given in Fig. 1. The proportion of very low density lipoprotein (VLDL) cholesterol to the total plasma cholesterol was significantly ($P < 0.05$) lower in rats fed the garlic extract, whole garlic, or autoclaved garlic diets than in animals fed the control diet. Just opposite results were obtained in the proportion of high-density lipoprotein (HDL) cholesterol to the plasma total cholesterol.

The supplementation of garlic extract, whole garlic, or autoclaved garlic also reduced the liver weight, inquinal fat pad weights, liver total lipid, and liver total and free cholesterol (Table II). Rats fed the diets added with garlic products except garlic residue decreased hepatic G-6-PDH and ME activities as compared with control animals.

Discussion. Garlic appeared as an effective agent to reduce blood cholesterol and triglycerides as well as liver lipids and cholesterol. The reduction in plasma cholesterol was in VLDL and LDL cholesterol as observed in a previous study (10). The VLDL cholesterol values (mean ± SD) for control, garlic residue, garlic extract, whole garlic, and autoclaved garlic groups were 34.3 ± 5.0, 31.0 ± 2.9, 18.4 ± 2.4, 20.0 ± 2.7, and 20.1 ± 2.6 mg/100 ml, respectively, and LDL cholesterol values (mean ± SD) for the same treatments were 37.3 ± 3.6, 35.5 ± 4.2, 26.1 ± 2.2, 27.8 ± 2.9, and 28.5 ± 3.7 mg/100 ml, respectively. Although the actual values of HDL cholesterol among treatment groups (31.2 ± 4.1, 31.2 ± 3.0, 30.1 ± 2.8, 30.0 ± 4.2, and 30.6 ± 2.8 mg/100 ml for control, garlic residue, garlic extract, whole garlic, and autoclaved garlic, respectively) were similar, the percentage of HDL cholesterol to the plasma total cholesterol was higher in animals fed the garlic products except garlic residue than the control group. This was because the plasma total cholesterol level was much lower in those garlic groups than in the control group. The importance of plasma cholesterol and lipoprotein concentrations in the pathogenesis of atherosclerosis has been noted by numerous investigators. Increased levels of total plasma cholesterol with increases in LDL or VLDL were associated with a greater risk of developing coronary heart diseases (1, 2) while high concentration of HDL appeared to be protective (18, 19). The changes in the proportions of lipoprotein cholesterol fractions may be one reason of protective effect of garlic against the atherosclerotic process.

G-6-PDH and ME activities have been shown to be correlated to lipogenic capacity in various tissues including rat liver (20) by supplying substrates for fatty acid synthesis (21). Garlic appeared to reduce fatty acid synthesis by decreasing key enzyme activities in supplying substrates and consequently reduced the lipid levels in the liver and plasma. The decrease in food intake in some of garlic-fed rats did not cause these metabolic changes since pair-fed animals with and without garlic in a previous

study (10) showed similar results as in the present study. The lesser weight gain in rats fed garlic products might be the result in part of decreases in food intake and in part of the metabolic changes due to garlic feeding. The observed metabolic changes in garlic-fed animals were not likely due to hepatic damage: the liver and gastrointestinal tract of rats were grossly examined for any tissue damage and found no visible tissue damage in controls and garlic groups.

Garlic was similarly effective in lowering cholesterol and lipid levels in the plasma as well as in the liver in a form of lyophilized whole garlic or ethanol extract of garlic suggesting that the active agent in garlic for reducing blood cholesterol was contained in the garlic extract. The nonsignificance of garlic residue, the portion after ethanol extract of whole garlic, for hypocholesterolemic activity also confirms the effectiveness of ethanol extract of garlic. Autoclaving the garlic at 120° for 1 hr did not diminish the hypocholesterolemic property of garlic.

1. Fredrickson DS, Levy RI, Lees RS. Fat transport and lipoproteins—An intergrated approach to mechanisms and disorders. N Engl J Med 276:32–35, 1967.
2. Stamler J. Epidemiology of coronary heart disease. Med Clin North Amer 57:5–46, 1973.
3. Gofman JW, Young W, Tandy R. Ischemic heart disease, atherosclerosis and longevity. Circulation 34:679–685, 1966.
4. Bordia A, Bansal HC, Arora SK, Singh SV. Effect of the essential oils of garlic and onion on alimentary hyperlipemia. Atherosclerosis 21:15–20, 1975.
5. Jain RC. Effect of garlic on serum lipids, coagulability and fibrinolytic activity of blood. Amer J Clin Nutr 30:1380–1381, 1977.
6. Kritchevsky D. Effect of garlic oil on experimental atherosclerosis in rabbits. Artery 1:319–323, 1975.
7. Bordia A, Verma SK, Vyas AK, Bhu N. Effect of essential oil of onion and garlic on experimental atherosclerosis in rabbits. Atherosclerosis 26:379–386, 1977.
8. Bordia A, Joshi HK, Sanadhya YK, Bhu N. Effect of essential oil of garlic on serum fibrinolytic activity in patients with coronary artery disease. Atherosclerosis 28:155–159, 1977.
9. Jain RC, Vyas CR, Mahatma OP. Hypoglycemic action of onion and garlic. Lancet 2:1491, 1973.
10. Chi MS, Koh ET, Stewart TJ. Effect of garlic on lipid metabolism in rats fed cholesterol or lard. J Nutr 112:241–248, 1982.
11. Bronzert TJ, Brewer HB. New micromethod for measuring cholesterol in plasma lipoprotein fractions. Clin Chem 23:2089–2096, 1977.
12. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509, 1957.
13. Kim E, Goldberg M. Serum cholesterol assay using a stable Liebermann–Burchard reagent. Clin Chem 15:1171–1179, 1969.
14. Kornberg A, Horecker BL. Glucose-6-phosphate dehydrogenase. In: Colowich SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 1:p323, 1955.
15. Ochoa S. Malic enzyme. In: Colowich SP, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol 1:p739, 1955.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–272, 1951.
17. Steel RGD, Torrie JH. Principles and Procedures of Statistics. New York, McGraw–Hill, p481, 1960.
18. Miller GJ, Miller NE. Plasma high density lipoprotein concentration and development of ischemic heart disease. Lancet 1:16–19, 1975.
19. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber, TR. High density lipoprotein as a protective factor against coronary heart disease. Amer J Med 62:707–714, 1977.
20. Tepperman HM, Tepperman J. Patterns of dietary and hormonal induction of certain NADP-linked liver enzymes. Amer J Physiol 206:357–362, 1964.
21. O'Hea EK, Leveille GA. Lipogenesis in isolated adipose tissue of the domestic chick. Comp Biochem Physiol 26:111–117, 1968.

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Survival of Porcine Embryos after Asynchronous Transfer[1-3] (41495)

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Abstract. Forty gilts and sows were used to transfer Day 5 embryos into one uterine horn, while Day 7 embryos were transferred into the other horn, of Day 6 nonpregnant recipients (Day 0 = first day of estrus). The survival of the transferred embryos was determined on Day 11 (Expt 1) and Days 60 to 70 (Expt 2). The percentage of Day 5 and 7 embryos surviving the transfer procedures on Day 11 was not different, 42 ± 10 and 43 ± 12, respectively. However, by midgestation (Day 60) more ($P < 0.001$) fetuses that developed from Day 7 embryos survived than fetuses that developed from Day 5 embryos, 63 ± 8 and 8 ± 7%, respectively. These experiments indicated that the presence of embryos more advanced in development caused the demise of younger embryos sometime between Days 11 and 60 of gestation.

Considerable variation exists in embryonic development within several polytocous species. This is not surprising because in pigs ovulation extends for a 6-hr period (1, 2). Accordingly, the first cleavage division of the fertilized ova occurs between 60 and 108 hr after the onset of estrus in sows (3). Anderson (4) noted marked variation in the morphology of porcine embryos between Days 11 and 13 of gestation. Within a uterine horn these embryos ranged in development from spherical and tubular to filamentous. Embryo survival was not altered when embryos were transferred 1 day from synchrony with the recipient (5). However, the biological significance of this normally occurring variation in embryonic development remains unclear.

Pigs naturally lose 40% of their embryos during gestation. Several physiological events, important for survival of the porcine embryo, are closely associated with morphological changes of the embryo. These events include estrogen synthesis (6), luteal maintenance (7), and transuterine migration of embryos (8). The possibility exists that embryos advanced in their development relative to their litter mates have a survival advantage.

The present experiment was conducted to determine if embryos more advanced in development migrated further within the uterine horns and had a greater chance for survival during pregnancy.

Materials and Methods. *Expt 1.* Sixteen cross-bred gilts and sows, checked daily for estrous behavior, were utilized in this experiment. Recipients, 6 days after the onset of estrus, received four to six embryos each from Day 5 and 7 donors (Day 0 = first day of estrus). Such a procedure allowed establishment of pregnancy with embryos 2 days apart in age but only 1 day from synchrony with the recipient. The uterine horn to which the embryos, within an age, were introduced was randomized. Gilts assigned on the appropriate days as donors were mated 4 and 24 hr after the onset of estrus. To maximize utilization of females, two recipients were used for each Day 5 and 7 donor. On Day 11, 5 days post-transfer, the recipients were slaughtered and the em-

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bryos recovered by flushing segments (10 to 20 cm) of the excised uterus with physiological saline. Age (Day 10 or 12) and location of the embryos and the length of uterine segments were recorded. The age of the recovered embryos was determined by morphology (spherical vs tubular or oblong) and size. Recovered embryos classified as originating from Day 5 donors, for example, were all spherically shaped, measuring 1 to 2 mm in diameter. Those identified as originating from Day 7 donors were spherical to tubular in shape, ranging from 4 to 15 mm in length. Only those paired recipients with recoverable embryos from each donor, as determined on Day 11 of gestation, were included in the statistical analysis.

The distance the embryos migrated (cm) was determined as follows: distance = {(sum of the length of uterine segments traversed by each embryo) − (the total number of Day 5 or 7 embryos transferred) × [the length of the anterior uterine segment (10 to 20 cm) containing the respective embryos]}. It was necessary to subtract the length of the anterior segments because the embryos were located in these segments before their migration. Distance the Day 5 or 7 embryos migrated was compared by using a least-squares analysis of variance.

Expt. 2. Twenty-four [eight purebred colored (Duroc, Spotted or Hampshire), eight purebred white (Yorkshire, Landrace or Large White) and eight crossbred] gilts and sows were utilized in this experiment. Day 6 recipients received embryos in accordance with procedures described in Expt 1 except donors were mated to colored or white boars. One recipient was used for each Day 5 and 7 donor. Attempts were made to balance the number of Day 5 and 7 embryos transferred into each recipient (10.9 ± 0.9 Day 5 and 10.4 ± 1.0 Day 7 embryos, $\bar{x}$ ± SE). Breed of donor (colored vs white) was randomized such that four Day 5 and four Day 7 colored donors were utilized. Fetuses were recovered between Days 60 and 70 of gestation, identified by skin pigmentation, weighed, and the distance between adjacent fetuses was noted. One gilt aborted on Day 60, in which case, all fetuses were recovered immediately and skin pigmentation was noted. Fetal weight was subsequently correlated with the distance between adjacent fetuses. The percentage of Day 5 and 7 embryos surviving to Day 11 (Expt 1) and 60 of gestation was compared by a nonparametric Mann–Whitney *U* test.

To confirm that Day 5 embryos can survive in Day 6 recipients in the absence of Day 7 embryos, 10 additional recipients received only Day 5 embryos (8.8 ± 0.7 embryos per uterine horn). Comparisons of the percentage of Day 5 and 7 embryos surviving to Day 60 (Expt 2) to the percentage of Day 5 embryos surviving to Day 60 alone were conducted by use of a nonparametric Mann–Whitney *U* test and unpaired *t* test, respectively.

Embryo manipulation. Embryos were collected surgically from donors by flushing the anterior half of each uterine horn toward a catheter (medical grade Teflon tubing, 1.50-mm i.d., 2.11-mm o.d.) located 1 to 2 cm posterior to the uterotubal junction. The flushing medium (Table I) was similar to that utilized by Davis and Day (9) except lactate and pyruvate were deleted; antibiotic–antimycotic was substituted for penicillin G and streptomycin, and sodium chloride increased to maintain physiological osmolarity. Recovered embryos were incubated (39°, 95% air–5% CO_2) for not more than 30 min before being transferred. This transfer procedure consisted of effluxing the embryos and medium (100 to 200 μl) into the uterine lumen (2 to 3 cm posterior to the uterotubal junction) through a catheter (medical grade Teflon tubing, 0.69-mm i.d., 0.99-mm o.d.) temporarily inserted through the posterior 3 to 4 cm of the oviduct. Only morphologically normal embryos were utilized. Transferable Day 5 embryos ranged from late compacted morula to blastocysts with a small to medium blastocoel and a recognizable inner cell mass. The Day 7 embryos had an expanded blastocoel and a comparably flattened inner cell mass and thinner zona pellucida.

Results and Discussion. Results of Expt 1 (Table II) indicated no difference in the ability of Day 5 and 7 embryos to survive

TABLE I. MODIFIED KREBS–RINGER BICARBONATE[a]

Ingredient	g/500 ml	m*M*
NaCl	3.500	119.78
KCl	0.178	4.78
$CaCl_2 \cdot 2H_2O$	0.125	1.71
KH_2PO_4	0.081	1.19
$MgSO_4 \cdot 7H_2O$	0.147	1.19
$NaHCO_3$	1.053	25.00
Glucose	0.500	5.56
Bovine serum albumin[b]	2.000	
Antibiotic–antimycotic[c]	50,000 units/5 ml	

[a] Davis and Day (1978).
[b] Pentex bovine albumin crystallized, Miles Laboratories.
[c] Antibiotic–antimycotic, lyophilized, Gibco Laboratories.

for 5 days after transfer. However, by midgestation (Day 60) more fetuses that developed from Day 7 embryos (Expt 2; Table II) survived than fetuses that developed from Day 5 embryos ($P < 0.001$). At Day 60 only two recipients had fetuses that developed from transferred Day 5 embryos. Only females that remained pregnant to Day 60 were included in the data of Expt 2. This increased the proportion of Day 7 embryos recovered at Day 60 as compared with Day 11 because the percentage of all transferred Day 5 vs Day 7 embryos surviving to Days 11 and 60 was 34 vs 44 and 4 vs 36, respectively.

More ($P < 0.01$) Day 5 embryos survived to Day 60 in the absence than in the presence (42 vs 8%) of Day 7 embryos (Table II). Webel *et al.* (5) observed an equivalent survival rate of embryos transferred one day from synchrony (49 to 53%). Alternatively, the percentage of Day 7 embryos surviving to Day 60 in the presence of Day 5 embryos was not different from the percentage of Day 5 embryos surviving to Day 60 alone (42 vs 63%, respectively). These observations confirm that each population of transferred embryos can survive alone but when forced to cohabit in the uterus, fewer younger embryos survived to Day 60.

Identification of embryos by size in Expt 1 was more subjective than skin pigmentation of the fetuses in Expt 2. Wright and Grammer (10) observed a 25-fold increase in protein content of the porcine embryos between Days 8 and 9 of gestation. This

TABLE II. PERCENTAGE SURVIVAL OF DAYS 5 AND 7 EMBRYOS TO DAYS 11 AND 60 OF GESTATION

			Day 5 embryos			Day 7 embryos		
	No. recipients utilized	No. recipients pregnant	No. embryos transferred	No. survived	Survival/ recipient (%)	No. embryos transferred	No. survived	Survival/ recipient (%)
Day 11	10	8[a]	45	19	42 ± 10[d]	42	18	43 ± 12[d]
Day 60	16	8[b]	87	6	8 ± 7[e]	83	53	63 ± 8[f]
Day 60	10	5[c]	88	35	42 ± 10			

[a] Two recipients were not included as 5 of the 0 transferred Day 7 embryos were recovered and none of the 11 Day 5 embryos.
[b] Eight recipients were not included after failing to maintain pregnancy following the introduction of a total of 78 Day 5 and 63 Day 7 embryos.
[c] Five recipients were not included after failing to maintain pregnancy following the introduction of a total of 89 Day 5 embryos.
[d,e,f] Means with different superscripts within rows are different ($P < 0.001$).

exponential growth of embryos continued between Days 9 and 16. Although considerable variation existed, spherical shaped Day 12 embryos were larger and contained fourfold more protein than spherical Day 10 embryos (4). Little difficulty was experienced in the present study in differentiating between the transferred Day 5 and 7 embryos on Day 11.

Transuterine migration of porcine embryos occurs between Days 7 and 12 (11). Recovered embryos 5 days post-transfer were mixed within both uterine horns as was previously observed with synchronous transfer (12). The older embryos (Day 12) failed to migrate further than the younger embryos (Day 10) when examined on Day 11, 160.1 ± 29.6 vs 113.5 ± 16.1 cm, respectively. However, the distance the embryos migrated may have been different if examined at an earlier time. Because only about 40% of the transferred embryos were viable on Day 11, differentiating the healthy from the dying embryos may have been difficult before this time.

Recovered fetuses ranged in weight from 60.1 to 274.5 g and crown–rump length from 7.5 to 19 cm. However, neither fetal weight nor length was associated with the age of the transferred embryo (172.7 ± 14.2 vs 157.0 ± 9.3 g and 14.5 ± 0.9 vs 13.9 ± 0.4 cm, Day 5 vs Day 7 embryos, respectively). After the variation in fetal weight between recipients was reduced by standardizing the weight of the heaviest fetus within each litter and then adjusting the weight of each remaining litter mates proportionately, the distance between adjacent fetuses was highly correlated with fetal weight ($r = 0.47$, $P < 0.01$). Rathnasabapathy *et al.* (13) observed a similar relationship with fetuses examined on Day 55 of gestation. Knight *et al.* (14) demonstrated a significant correlation of fetal weight to placental length suggesting a relationship between migration of the porcine embryo, outgrowth of the placenta, and the subsequent development of the fetus.

Although the majority of embryonic loss occurs by Day 30 (15–19) it is unknown when or why the young embryos (Day 5) died between Days 11 and 60 of gestation. Because the porcine embryo can elongate rapidly, 3 cm/hr (20), the possibility exists that older embryos (Day 7) elongated sooner and occupied more of the uterus than the younger (Day 5) embryos. Anderson (4) observed the inability of embryos to overlap each other regardless of the uterine space available. Knight *et al.* (14) observed an increase in mortality of crowded fetuses between Days 40 and 100 and suggested this was due to placental insufficiency. Perhaps in this experiment the younger embryos (Day 5) died because of placental insufficiency as a result of crowding by the older embryos (Day 7).

Another explanation for the loss of the younger embryos (transferred at Day 5) might include physiological advancement in the biochemical development of the recipient's uterus such that the younger embryos could no longer continue to develop. Beier *et al.* (21) and Adams (22) demonstrated the fragile relationship between synchronizing the pattern of uterine secretions and the age of successfully transferred rabbit embryos. Exogenous estrogen extends the length of the estrous cycle of nonpregnant pigs (23) possibly by altering secretion of uterine proteins (24), intrauterine sequestering of prostaglandin (25), and/or uterine blood flow (26). It is possible the older embryos (Day 7), by synthesizing estrogen earlier, advanced the secretory pattern(s) of the uterus resulting in the demise of the younger embryos (Day 5).

The precise mechanism by which some embryos survive and others die in polytocous species is unclear. These experiments indicated porcine embryos more embryologically advanced have a greater chance to survive and may have caused the demise of those less embryologically developed.

1. Burger JF. Sex physiology of pigs. Onderstepoort J Vet Res 25(Suppl 2):1–218, 1952.
2. Lewis LL. The vitality of reproductive cells. Okla Agric Exp Stn Bull 96, 1911.
3. Oxenreider SL, Day BN. Transport and cleavage of ova in swine. J Anim Sci 24:413–417, 1965.
4. Anderson LL. Growth, protein content and dis-

tribution of early pig embryos. Anat Rec 190:143–153, 1978.
5. Webel SK, Peters JB, Anderson LL. Synchronous and asynchronous transfer of embryos in the pig. J Anim Sci 30:565–568, 1970.
6. Ford SP, Christenson RK, Ford JJ. Uterine arterial blood flow and uterine luminal content and secretion of oestrone and oestradiol-17β on Days 11, 13, and 15 of the oestrous cycle and gestation of sows. J Reprod Fertil 64:185–190, 1981.
7. Frank M, Bazer FW, Thatcher WW, Wilcox CJ. A study of prostaglandin $F_{2}\alpha$ as the luteolysin in swine. III. Effects of estradiol valerate on prostaglandin F, progestins, estrone and estradiol concentrations in the utero-ovarian vein of nonpregnant gilts. Prostaglandins 14:1183–1196, 1977.
8. Patten BM. Embryology of the Pig, 3rd ed. Blakiston, Philadelphia, 1948.
9. Davis DL, Day BN. Cleavage and blastocyst formation by pig eggs *in vitro*. J Anim Sci 46:1043–1053, 1978.
10. Wright RW, Jr, Grammer JC. Size variation and total protein in porcine embryos collected from individual pigs. Theriogenology 13:111, 1980.
11. Dhindsa DS, Dziuk PJ, Norton HW. Time of transuterine migration and distribution of embryos in the pig. Anat Rec 159:325–330, 1967.
12. Dziuk PJ, Polge C, Rowson LE. Intra-uterine migration and mixing of embryos in swine following egg transfer. J Anim Sci 23:37–42, 1964.
13. Rathnasabapathy V, Lasley JF, Mayer DT. Genetic and environmental factors affecting litter size in swine. MO Agric Exp Stn Bull 615, 1956.
14. Knight JW, Bazer FW, Thatcher WW, Franke DE, Wallace HD. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci 44:620–637, 1977.
15. Longenecker DE, Day BN. Fertility level of sows superovulated at post weaning estrus. J Anim Sci 27:709–711, 1968.
16. Pope CE, Vincent CK, Thrasher DM. Effect of I.C.I. 33,828 and PMS on reproduction in gilts. J Anim Sci 27:303A, 1968.
17. Pope CE, Christenson RK, Zimmerman-Pope VA, Day BN. Effect of number of embryos on embryonic survival in recipient gilts. J Anim Sci 35:805–808, 1972.
18. Bazer FW, Robison OW, Clawson AJ, Ulberg LC. Uterine capacity at two stages of gestation in gilts following embryo superinduction. J Anim Sci 29:30–34, 1969.
19. Webel SK, Dziuk PJ. Effect of stage of gestation and uterine space on prenatal survival in the pig. J Anim Sci 38:960–963, 1974.
20. Geisert RD, Bazer FW, Brookbank JW. Morphological changes association with porcine blastocyst elongation. J Anim Sci 53(Suppl 1):320A, 1981.
21. Beier HM, Mootz U, Kuhnel, W. Asynchronous egg transfer during delayed secretion in the rabbit. VII. Inst. Congress for Animal Reproduction, Munich, Vol 3:pp1891–1896, 1972.
22. Adams CE. Asynchronous egg transfer in the rabbit. J Reprod Fertil 35:613–614, 1973.
23. Gardner ML, First NL, Casida LE. Effect of exogenous estrogen on corpus luteum maintenance in gilts. J Anim Sci 22:132–138, 1963.
24. Geisert RD, Bazer FW, Basha SMM, Roberts RM. Quantitative and qualitative aspects of uterine protein secretions from pseudopregnant and unilaterally pregnant gilts. J Anim Sci 49(Suppl 1):299A, 1979.
25. Frank M, Bazer FW, Thatcher WW, Wilcox CJ. A study of prostaglandin $F_{2}\alpha$ as the luteolysin in swine. IV. An explanation for the luteotrophic effect of estradiol. Prostaglandins 15:151–159, 1978.
26. Ford SP, Magness RR. Effect of intra-uterine infusion of estradiol-17β ($E_{2}\alpha$) on luteal function in nonpregnant sows. J Anim Sci 51(Suppl 1):279, 1980.

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Evidence for an Intestinal Factor Stimulating Hepatic Cholesterogenesis (41496)

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Abstract. External diversion of bile leads to an increase in hepatic cholesterol synthesis. To study the role of the small intestine in this phenomenon we conducted a series of experiments in bile-diverted rats with and without surgical removal of most of the small intestine, its upper half or its lower half. The pancreas was preserved intact in all experiments. Hepatic cholesterol synthesis at the time of surgery and 24 hr later was measured in liver homogenates using [2-^{14}C]acetate as substrate. Hepatic cholesterogenesis increased almost 4-fold 24 hr after biliary diversion in rats with intact intestine, and decreased to 64% of the baseline rate in bile-diverted rats with the jejunum–ileum removed, and to 58% in sham-operated animals. To investigate the possibility that the stimulation seen in the bile-diverted rats with intact intestine was due to a substance absorbed from the diet, the experiments were repeated with animals fed only 10% glucose in water 24 hr prior to surgery. Again the rats with intact small intestine showed an increase in hepatic cholesterogenesis (6.7-fold) while those with the jejunum-ileum removed and the sham operated showed a decrease to 60 and 70% of the baseline rate, respectively. These results support the hypothesis that the small intestine produces a factor(s) that stimulates hepatic cholesterol synthesis in response to a drop in the intraluminal content of bile. To narrow the site of production of the factor(s), the effect on hepatic cholesterogenesis of removal of the upper or lower half of the small intestine in bile diverted animals was studied by comparison to a group of bile-diverted intestine-intact controls. As before, the latter showed a 6-fold increase in hepatic cholesterogenesis. With the upper or lower half of the small intestine removed a similar degree (4- to 5-fold) of stimulation was still observed. These results suggest that the factor(s) is produced along the entire length of the jejunum-ileum.

On the basis of a substantial body of suggestive evidence Krumdieck and Ho (1) have postulated that the intestine plays a role in the regulation of hepatic cholesterogenesis by producing a stimulatory factor whenever the intraluminal concentration of bile acids drops below requirements. The factor is presumably transported to the liver by the portal circulation. According to this hypothesis, the well-documented increase in the rate of hepatic cholesterol synthesis seen in animals in which the flow of bile has been diverted away from the intestinal lumen by means of a biliary fistula (2) would be the result of an increased production of the intestinal stimulatory factor. Conversely, it may be postulated that the intestine produces an inhibitory factor of hepatic cholesterogenesis, the amount of which is decreased when the concentration of bile in the lumen decreases. If this hypothesis is true the functional integrity of both the intestine and the portal circulation would be essential for the elevation of hepatic cholesterogenesis in bile-diverted animals. Consequently, removal of the small intestine from a bile-diverted animal ought to completely eliminate the normally observed elevation of the rate of cholesterol synthesis in the liver. In this article we report the effects on hepatic cholesterogenesis of the simultaneous production of a bile fistula and the removal of nearly all the small intestine (from the ligament of Treitz to the cecum), its upper half, or its lower half, in rats. The results obtained support the existence of the proposed intestinal stimulatory factor.

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Materials and Methods. *Animals and surgical procedures.* Male Sprague–Dawley rats weighing 200 to 250 g were used in all the experiments. The animals were maintained in an air-conditioned room with alternating 12-hr light and dark cycles; the lights were on from 6:00 AM until 6:00 PM. To minimize confounding effects attributable to the circadian rhythm of hepatic cholesterol synthesis all animals were operated and sacrificed between 9:00 and 10:00 AM. The animals were maintained for at least one week prior to surgery on a modified MIT-200 diet (3) containing by weight 20% casein, 65% sucrose, 5% corn oil, 2% agar, and 4% cellulose plus 1% vitamin mix and 3% salt mixture. Under ether anesthesia groups of animals were subjected to one of the following surgical procedures:

(*a*) *Liver sampling and biliary diversion:* After ligating the pedicle, the lateral portion of the medial lobe of the liver was excised and immediately homogenized as described below to determine the baseline rate of hepatic cholesterol synthesis. After removal of the liver sample the common bile duct was cannulated using a polyethylene tube (PE-10; o.d. 0.6 mm) which was externalized through a small orifice in the abdominal wall and securely tied to the skin.

(*b*) *Liver sampling, biliary diversion, and removal of the small intestine:* The animals in this group underwent the same procedures to remove a sample of liver and to establish a biliary fistula as those in group (a). In addition, most of the small intestine was removed along its mesenteric attachment leaving in place the duodenum and approximately 1 cm of the distal ileum. The duodenum and terminal ileum were connected with a short plastic tube (o.d. 4 mm) to prevent the development of gastric distention. This operation leaves the pancreas intact and does not alter the venous drainage of the pancreatic circulation into the portal system.

(*c, d*) *Liver sampling, biliary diversion, and removal of either the upper* (*c*) *or the lower* (*d*) *half of the small intestine:* After sampling the liver and preparing a biliary fistula as in group (a), either the upper or the lower half of the small intestine was excised. When removing the upper half, the duodenum and 1 cm of the proximal jejunum were left in place. The continuity of the intestine was restored as in procedure (b) by means of a plastic tube. The same was done when removing the lower half of the intestine connecting the stump of terminal ileum to the remaining upper half of the intestine.

(*e*) *Liver sampling and sham-operation:* The animals in this group underwent removal of the lateral portion of the medial lobe of the liver followed by manipulation of the viscera to mimic the surgical trauma of the other groups. No bile fistula was prepared in this group.

All animals received a subcutaneous injection of 20 ml of 5% glucose in saline immediately after surgery and were maintained at room temperature and fasted for the next 24 hr following which they were sacrificed and their livers removed for estimation of the rate of cholesterol synthesis.

Determination of the rate of cholesterol synthesis. The samples of liver removed at the time of surgery and at the time of sacrifice were washed in ice-cold Tris–HCl buffer, 0.1 *M* pH 7.8, containing 0.01 *M* K_2HPO_4, 0.03 *M* nicotinamide, 0.1 m*M* EDTA, and 0.6 m*M* $MgCl_2$. The pieces were then blotted dry, weighed, and homogenized in the above buffer (2.5 ml/g of liver) containing also 5.0 m*M* oxidized glutathione. The homogenization was done using a loosely fitting glass–Teflon Potter-Elvehjem homogenizer and was completed in two strokes. The homogenates were centrifuged at 500*g* for 20 min at 4°. Eight-tenths milliliter of supernatant was incubated at 37° in a Dubnoff incubator (140 oscillations/min) for 5 min to allow temperature equilibration and the synthesis was initiated by the addition of 0.2 ml of a solution containing 1.8 m*M* [2-^{14}C]sodium acetate (specific activity, 0.4 mCi per mmole), coenzyme A 50 μM, ATP 1.0 m*M*, NADP 0.5 m*M*, and glucose 6-phosphate 3.0 m*M*. The incubation was continued for 30 min at the end of which 2.0 ml of 95% ethanol and 0.5 ml of 60% KOH (w/v) were added. After saponification for 90 min at 8[illegible] the 3-β-hydroxy steriods were precipitat[illegible]

as the digitonides, washed, and counted as described before (4). Protein determinations in the homogenates were done following the Lowry method with bovine albumin as standard (5). The results are given in terms of nanomoles of acetate incorporated into cholesterol per gram of protein per hour. The percentage change in cholesterol synthesizing activity between the baseline and the 24-hr post-surgery values were calculated for each animal.

Results. *Effect of biliary diversion on the rate of hepatic cholesterogenesis 24 hr after surgery.* Before studying the effect of intestinal removal upon the hepatic cholesterol synthetic activity of bile fistula rats, it was necessary to demonstrate that the stimulation of hepatic cholesterogenesis after bile diversion occurred within a period short enough to assure the survival of the animals with the small intestine removed. Penhos *et al.* (6) demonstrated that eviscerated rats with a functional liver in which the stomach, small and large intestines, pancreas, mesentery and spleen had been excised and in which a bile fistula had been produced to allow continuity of bile secretion, survived for more than 72 hr provided that dehydration was prevented by the administration of saline. It was therefore safe to assume that rats subjected to the much less traumatic procedure (b), i.e., liver sampling, biliary diversion, and excision of the small intestine, would easily survive for 24 hr. It remained to be demonstrated that 24 hr after biliary diversion there was already a detectable increase in the rate of hepatic cholesterogenesis. To this effect three rats, fed the maintenance diet until the time of the experiment, were subjected to procedure (a), i.e., liver sampling and biliary diversion, and the rate of hepatic cholesterol synthesis was determined at the time of surgery and 24 hr later. In these animals the incorporation of acetate into cholesterol increased significantly from 198 ± 12 (mean ± SE) nmole/g of protein/hr to 552 ± 8 nmole/g protein/hr, or to 280 ± 30% of the baseline value ($P < 0.001$).

Effect of removal of the small intestine on the rate of hepatic cholesterol synthesis of rats 24 hr after total biliary diversion: Experiment I. Six animals were subjected to procedure (a), liver sampling and biliary diversion with intact intestine; five to procedure (b), liver sampling, biliary diversion, and nearly total removal of the small intestine; and four to procedure (e), liver sampling and sham operation. All these animals were fed the maintenance diet until the day of the experiment. The results are summarized in Table I. As in the preliminary experiment, biliary diversion markedly increased the rate of hepatic cholesterol synthesis to 377 ± 65% of the baseline value ($P < 0.01$). Removal of the small intestine in the bile-fistula rats completely eliminated this response resulting instead, in a small but significant drop of hepatic cholesterol synthetic activity to 64 ± 10% of the baseline rate ($P < 0.01$). The sham-operated animals showed also a modest but significant ($P < 0.05$) decrease in the rate of hepatic cholesterogenesis (Table I).

Experiment II. To investigate the possibility that the stimulation seen in the bile-diverted rats with intact intestine was due to some substance absorbed from the diet, the experiment was repeated with animals that consumed the maintenance diet until 24 hr prior to surgery and then given 10% glucose in water *ad libitum* as their only food. The results are shown in Table I. As in experiment I, cholesterol synthesis increased very significantly (to 670 ± 79% of baseline, $P < 0.01$) in the bile-diverted rats and decreased slightly (to 60 ± 8% of the baseline ($P < 0.01$) in the bile-diverted animals with the jejunum and ileum removed. The sham-operated group also showed a small decrease in hepatic cholesterogenesis. The most striking difference between experiments I and II was in the absolute rate of cholesterol synthesis which was many times higher in the diet-fed than in the glucose-fed group.

Effect of partial removal of the small intestine on the rate of hepatic cholesterol synthesis of rats 24 hr after total biliary diversion. Experiments I and II support the existence of a stimulatory factor of hepatic cholesterol synthesis produced by the jejunum-ileum. In an attempt to determine more precisely the portion of the small in-

TABLE I. EFFECT OF REMOVAL OF THE SMALL INTESTINE[a] ON THE RATE OF HEPATIC CHOLESTEROL SYNTHESIS OF RATS 24 HR AFTER TOTAL BILIARY DIVERSION

Surgical procedure	Experiment I (fed maintenance diet until surgery)			Experiment II (fed 10% glucose 24 hr prior to surgery)		
	Baseline rate	24 hr after surgery		Baseline rate	24 hr after surgery	
		Rate[b]	% of baseline rate		Rate[b]	% of baseline rate
Biliary diversion with intact intestine	88 ± 35 (n = 6)[c]	293 ± 120*	377 ± 65†	3.2 ± 0.7 (n = 6)	21.4 ± 5.5†	670 ± 79†
Biliary diversion and removal of the small intestine	124 ± 64 (n = 5)	75 ± 37*	64 ± 10†‡	3.5 ± 1.3 (n = 5)	2.4 ± 0.8*‡	60 ± 8†‡
Sham operation. Intact biliary tract and intestine	101 ± 56 (n = 4)	68 ± 51*	58 ± 12*‡	2.0 ± 0.9 (n = 4)	1.4 ± 0.9*‡	70 ± 8†‡

Note. No difference found between the group with biliary diversion and removal of the small intestine and the sham-operated group.

[a] From the ligament of Treitz to the cecum.

[b] Mean ± SE nmole of [2-^{14}C]acetate incorporated into cholesterol/g of protein/hr.

[c] Number of animals.

*,† Significant difference from the baseline rate by paired t test at $P < 0.05$ (*) or $P < 0.01$ (†).

‡ Significant difference from the group with biliary diversion and intact intestine by Student's t test at $P < 0.01$.

testine involved in its production, biliary-diverted rats with either the upper half or the lower half of the small intestine removed were prepared and the rate of cholesterol synthesis of their livers before and after surgery was compared to that of biliary-diverted animals with the small intestine intact. All the animals used in this experiment were fed nothing but 10% glucose *ad libitum* 12 hr prior to surgery. The results are shown in Table II. It can be seen that preserving either the upper or the lower half of the small intestine was sufficient to elicit the increase in the rate of hepatic cholesterogenesis characteristic of bile-fistula rats with intact small intestine (Table II). Although the extent of the stimulation found in the animals with partial removal of the small intestine seemed to be lower than in the intact animals, no statistically significant difference could be demonstrated among the three groups.

Discussion. It is well established that removal of bile from the intestinal lumen, by whatever means, results in increased rates of hepatic cholesterol synthesis. Thus, ligation of the bile duct (7), creation of a bile fistula (2), cholestyramine administration (8), or ileal bypass (9), all produce significant elevations in hepatic cholesterogenesis. We have postulated (1) that these responses follow a homeostatic mechanism, mediated by a stimulatory factor produced by the intestine, and serving to restore to normal the intraluminal concentration of bile acids whenever it drops below requirements. The stimulation of *de novo* hepatic bile acids synthesis implies a stimulation of the biosynthesis of cholesterol, their obligatory precursor. This hypothesis is supported by the results presented in Table I, experiment I. The complete disappearance of the stimulation of liver cholesterogenesis in the bile-fistula rats when the jejunum-ileum was removed argues strongly for the loss of a stimulatory factor or factors contributed by the small intestine.

The presence in portal blood of a substance or substances capable of promoting higher rates of hepatic cholesterol synthesis has been indicated before by the elegant experiments of Starzl *et al.* involving partial transposition of portal and vena caval blood in dogs (10, 11). In these studies, portal blood was supplied to one portal branch of the liver while the other portal branch was supplied with blood from the inferior vena cava. The lobes receiving the portal blood had higher cholesterol synthesizing activity than the lobes irrigated with systemic blood. Starzl and his co-workers attributed this difference primarily, but not solely, to the much higher concentration of insulin in portal than in caval blood. Although the role of insulin in maintaining the trophism

TABLE II. EFFECT OF PARTIAL REMOVAL OF THE SMALL INTESTINE ON THE RATE OF HEPATIC CHOLESTEROL SYNTHESIS OF RATS 24 HR AFTER TOTAL BILIARY DIVERSION

Surgical procedure	Baseline rate	24 hr after surgery	
		Rate[a]	% of baseline rate
Biliary diversion with intact intestine (n = 4)[b]	42 ± 6	258 ± 49*	615 ± 78†
Biliary diversion and removal of the upper half of the small intestine (n = 4)	45 ± 11	182 ± 39*	409 ± 57†
Biliary diversion and removal of the lower half of the small intestine (n = 5)	59 ± 10	303 ± 56†	511 ± 36†

Note. No significant differences found between the three groups of rats either in baseline rates or rates after surgery.

[a] Mean ± SE nmole of [2-^{14}C]acetate incorporated into cholesterol/g of protein/hr.

[b] Number of animals.

*,† Significant difference from the baseline rate by paired t test at $P < 0.01$ (*) or $P < 0.001$ (†).

of the liver cannot be denied, it is difficult to attribute our findings to differences in the supply of insulin to the liver of the bile-diverted animals with and without small intestine since in all of our groups the pancreas and its venous drainage into the portal vein were preserved intact. Furthermore, all animals were fasted after surgery which should have reduced the concentration of insulin in the portal blood to very low levels in all of our groups. Schneider *et al.* (12) have also provided experimental support for the existence of a factor in blood capable of stimulating liver cholesterol synthesis. By cross-circulation studies they showed that the blood of ileal bypassed rats stimulated hepatic cholesterol synthesis in their intact parabiosed partners. The ileal bypass procedure employed by these authors diverts the flow of bile from the distal half of the small intestine which, according to our hypothesis, would then respond by producing the postulated stimulatory factor.

A slight, but significant decrease of hepatic cholesterol synthetic activity was observed in the sham-operated animals and in the group with biliary diversion and jejunum-ileum removal. This effect is attributed to the period of fasting (13) after surgery. The most important difference, however, was the total disappearance of the stimulation of hepatic cholesterogenesis in the bile-diverted animals with simultaneous removal of the jejunum-ileum. These results are compatible with the postulate that a stimulatory factor is produced by the intestine in response to decreased intraluminal concentrations of bile. The alternative hypothesis, that the intestine generates an inhibitor of liver cholesterol synthesis which would be produced in lesser quantities in the bile-diverted animals, can safely be discarded since this explanation implies a maximal rate of cholesterol synthesis in the animals with the small intestine removed in which production of the inhibitory factor would have dropped to near zero.

Experiment II was an attempt to answer the question of whether or not the intestinal factor(s) is produced by the intestine or is absorbed from the lumen where it may be present as a dietary component or as a constituent of the secretions of the digestive apparatus. We reasoned that if a similar stimulatory response to that observed in experiment I was obtained when the animals had received nothing but 10% glucose in water for 24 hr prior to surgery, it could not be attributed to something absorbed from the diet. Although our results show comparable percentage increases in the rates of cholesterol synthesis of the bile-diverted intestine-intact animals in both experiments I and II, and the total abolition of this response by intestinal removal, the baseline rates of cholesterogenesis in experiment II were strikingly lower than those of experiment I. We attribute this to the effect of food deprivation in experiment II. Fasting markedly inhibits the rate of hepatic cholesterol synthesis (13) and we have calculated that the rats fed 10% glucose as their sole food for 24 hr prior to surgery (experiment II) consumed during that period less than 10% of the calories ingested by the animals fed the maintenance diet until the time of surgey (experiment I). Furthermore, the rats subjected to partial intestinal removal (vide infra) which were fed nothing but 10% glucose for 12 hr prior to surgery had intermediate baseline rates of liver cholesterogenesis, as would be expected given their shorter period of food restriction. Taken together the results of experiments I and II indicate that the baseline rate of hepatic cholesterogenesis, set as a function of recent food intake, can be accelerated about three- to sevenfold following bile diversion. The intestinal stimulatory factor appears therefore capable of altering the baseline rate but is certainly not its sole determinant. Furthermore, the results of experiment II support the contention that the factor is produced by the intestine and not merely absorbed from the luminal content.

Removal of either the upper or lower half of the jejunum-ileum failed to prevent the stimulation of hepatic cholesterogenesis after creation of a bile fistula (Table II). These results indicate that the small intestine

has a considerable reserve capacity for production of the stimulatory factor, and that this function resides in both the lower and upper halves of the jejunum-ileum. It seems clear also that the duodenum lacks the capacity to produce the stimulatory factor. These experiments also indicate that the absence of stimulation of cholesterogenesis observed in the bile-fistula animals with removal of the entire jejunum-ileum cannot be attributed to the surgical trauma per se. There is little difference in the trauma inflicted to the animals undergoing removal of half or the whole of the jejunum-ileum.

Based on the data provided by our experiments we conclude that removal of bile (or more precisely of bile acids) from the intestinal lumen leads to the production by both the upper and lower halves of the jejunum-ileum of a stimulatory factor or hormone which reaches the liver via the portal circulation and accelerates the rate of hepatic cholesterogenesis. The isolation and characterization of such a hormone should advance our understanding of the physiological regulatory mechanisms of cholesterol homeostasis.

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1. Krumdieck CL, Ho KJ. Intestinal regulation of hepatic cholesterol synthesis: an hypothesis. Amer J Clin Nutr 30:255–261, 1977.
2. Myant NB, Eder HA. The effect of biliary drainage upon the synthesis of cholesterol in the liver. J Lipid Res 2:363–368, 1961.
3. Navia JM, Lopez H, Harris RS. Purified diet for dental caries research with rats. J Nutr 97:133–140, 1969.
4. Siperstein MD, Guest MJ. Studies on the site of the feedback control of cholesterol synthesis. J Clin Invest 39:642–652, 1960.
5. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
6. Penhos JC, Woodbury C, Tizabi Y, Ramey ER. Metabolic studies in eviscerated rats with functional livers. Proc Soc Exp Biol Med 148:1159–1163, 1975.
7. Fredrickson DS, Loud AV, Hinkelman BT, Schneider HS, Frantz Jr ID. The effect of ligation of the common bile duct on cholesterol synthesis in the rat. J Exp Med 99:43–53, 1954.
8. Huff JW, Gilfillan JL, Hunt VM. Effect of cholestyramine, a bile acid binding polymer on plasma cholesterol and fecal bile acid excretion in the rat. Proc Soc Exp Biol Med 114:352–355, 1963.
9. Moutafis CD, Myant NB. Increased hepatic synthesis of cholesterol after ileal by-pass in monkeys. Clin Sci 34:541–548, 1968.
10. Starzl TE, Lee IY, Porter KA, Putnam CW. The influence of portal blood upon lipid metabolism in normal and diabetic dogs and baboons. Surg Gynecol Obstet 140:381–396, 1975.
11. Starzl TE, Porter KA, Francavilla JA, Benichou J, Putnam CW. A hundred years of the hepatotrophic controversy. In: Hepatotrophic Factors. Ciba Foundation Symposium 55 (new series). Amsterdam, Elsevier, pp111–129, 1978.
12. Schneider PD, Guzman IJ, Rucker RD, Stocks TG, Varco RL, Buchwald H. Increased hepatic cholesterol synthesis in normal rats by cross-circulation with ileal bypassed partners. Atherosclerosis 34:383–389, 1979.
13. White LW, Rudney H. Regulation of 3-hydroxy-3-methylglutarate and mevalonate biosynthesis by rat liver homogenates. Effects of fasting, cholesterol feeding, and Triton administration. Biochemistry 9:2725–2730, 1970.

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Response of the Renal Vitamin D Endocrine System to Oxidized Parathyroid Hormone (1-34) (41497)

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Abstract. Two preparations of bovine parathyroid hormone (bPTH), the natural bPTH(1-84) and the synthetic bPTH(1-34) fragment, have been treated with hydrogen peroxide and assayed for the effect of such treatment on the renal vitamin D endocrine system in Japanese quail. The oxidized and untreated preparations were injected intramuscularly into 4-week-old male Japanese quail, 12 hr after which the kidneys were removed and homogenized. The kidney homogenates were incubated with tritiated 25-hydroxyvitamin D_3[25-(OH)D_3] and the production rates of 1,25-$(OH)_2D_3$ and of 24,25-$(OH)_2D_3$ were determined as indices of 25-(OH)D_3-1-hydroxylase and 25-(OH)D_3-24-hydroxylase activities, respectively. Both untreated bPTH(1-34) and untreated bPTH(1-84) stimulated 1-hydroxylase and suppressed 24-hydroxylase activities. Oxidation of either bPTH(1-34) or bPTH(1-84) did not eliminate these responses, whereas the effects of oxidation on other responses to bPTH(1-34), namely inactivation of the hypotensive and renal adenylate cyclase stimulating responses, were observed as anticipated from our earlier observations. The importance of these findings is heightened when viewed in the context of our previous reports that oxidation of bPTH(1-34) leaves the hypercalcemic and hypocalciuric responses intact while partially or possibly totally inactivating all other major responses studied to date. It may be concluded that the mechanisms involved in effecting the hypercalcemic, hypocalciuric, and renal 25-(OH)D_3-1-hydroxylase responses to bPTH(1-34) demand structural requirements in the peptide molecule which are different from those needed to effect the hyperphosphaturic, hypophosphatemic, hypotensive, smooth muscle relaxing, and renal adenylate cyclase responses.

It is well known that the hypercalcemic activity of intact bovine parathyroid hormone [bPTH(1-84)] is largely inactivated by mild oxidation with hydrogen peroxide (1, 2). Recently, using conditions known to substantially inactivate the hypercalcemic activity of bPTH(1-84) in Japanese quail, we have shown that the synthetic fragment, bPTH(1-34), resists such inactivation with hydrogen peroxide (3, 4). Further work from our laboratory has revealed that, although the avian hypercalcemic activity of the synthetic bPTH(1-34) is unaffected, the hypotensive (4), hyperphophaturic (5), and uterine relaxing (6) activities of bPTH(1-34) are reduced by oxidation with hydrogen peroxide.

We now wish to report the effect of mild oxidation on the ability of intact bPTH(1-84) and of synthetic bPTH(1-34) to modify the renal 25-hydroxyvitamin D_3-1-hydroxylase and 25-hydroxyvitamin D_3-24-hydroxylase activities in Japanese quail. The untreated and oxidized preparations were also monitored for hypercalcemic, hypotensive, and renal adenylate cyclase stimulating activities.

Materials and Methods. *Hormone preparations.* Synthetic bovine parathyroid hormone tetratricontapeptide, bPTH(1-34), was obtained either from Beckman (Beckman Instruments, Inc., Bioproducts Operation, Palo Alto, Calif.; lot No. B01130) or from Peninsula (Peninsula Laboratories, Inc., San Carlos, Calif.; lot No. 001752). The bPTH(1-34) preparations had assigned potencies of 6000 and 10,000 IU/mg, respectively, when assayed *in vitro* using activation of rat renal cortical adenylate cyclase as the response. The intact bovine parathyroid hormone, bPTH(1-84), was purified material (N1BSC 77/533) kindly supplied by Dr. Joan Zanelli of the National

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Institute for Biological Standards and Control, London. It had an assigned potency of 2300 IU/mg.

Hydrogen peroxide treatment. One milliliter of a solution of either bPTH(1-84) or bPTH(1-34), each dissolved in acid saline (0.154 *M* NaCl adjusted to pH 3.0 with HCl) at a concentration of 1000 IU/ml, was treated with 0.3% hydrogen peroxide by adding 10 μl of 30% hydrogen peroxide (Fisher Scientific Co., Pittsburgh, Pa.; catalog No. H-325) to the 1.0-ml sample. Following addition of hydrogen peroxide, the mixture was incubated for 30 min at 25°. Control incubations were performed using 10 μl of water instead of the 30% hydrogen peroxide. The mixture was immediately lyophilized following incubation. The lyophilized preparations were sealed and stored below 0° until required. They were reconstituted in 1.0 ml of water and diluted in acid saline for injection. Total enzymatic digestion of the bPTH(1-34) preparation, subjected to oxidation under these conditions, has revealed that such treatment is 100% effective by reducing the methionine content from 1.74 moles per mole of peptide to zero (4). The evidence also indicated that the methionine is oxidized mole for mole to methionine sulfoxide. No other amino acid modifications were detected.

Renal 1- and 24-hydroxylase assays in Japanese quail. Four-week-old immature male Japanese quail (*Coturnix coturnix japonica*), weighing approximately 100 g and bred and maintained in the vivarium of the Texas Tech University Health Sciences Center as described elsewhere (7), were used. The control or bPTH solutions were injected intramuscularly in a volume of 0.4 ml/bird to five groups of five birds each: (a) control, acid saline; (b) bPTH(1-34); (c) oxidized bPTH(1-34); (d) bPTH(1-84); and (e) oxidized bPTH(1-84). Twelve hours after injection, the birds were sacrificed at which time the kidneys were removed for assessment of 1- and 24-hydroxylase activities *in vitro* as described elsewhere (8, 9). Each kidney homogenate was incubated for 10 min in the presence of 50 pmole of 25-[26,27-^{3}H]hydroxyvitamin D_3 with a specific activity of 9.6 Ci/mmole. The incubation mixture was extracted with appropriate solvents and the extract was dried, dissolved in 300 μl of chloroform/hexane (65:35), and applied to a 0.7 × 18-cm Sephadex LH-20 column. The column was eluted with the same solvent system and 50 fractions (125 drops or 0.92 ml each) were collected. The tritium content of each fraction was determined by liquid scintillation counting. Total disintegrations per minute (dpm) under each peak was calculated and corrected for extraction losses and the data were expressed as picomoles min^{-1} g^{-1} kidney. The 1,25-$(OH)_2D_3$ peak generated by Japanese quail kidney homogenates under these circumstances has failed to reveal any cochromatographic contaminants when subjected to more rigorous analysis (10).

Hypercalcemic activity in Japanese quail. The bPTH preparations were assayed for hypercalcemic activity in Japanese quail by the method described by Dacke and Kenny (2). All injections were given intravenously in a volume of 0.4 ml/bird using an injection vehicle consisting of 51 m*M* $CaCl_2$ and 0.01% bovine serum albumin.

Renal adenylate cyclase activity. A renal membrane preparation of adenylate cyclase was made from Japanese quail kidneys and used for the *in vitro* assay of the bPTH peptides. Details of the protocol, which was modified from assays using rat renal cortical membrane preparations (11–13), will be supplied on request. The assay is sensitive to about 0.01 μg/tube (2×10^{-8} *M*) of synthetic bPTH(1-34).

Hypotensive activity in the rat. The hypotensive effect of the bPTH peptides was assayed in the rat following intravenous injection as described elsewhere (14). A significant hypotensive response may be seen with 1 μg/kg. A typical response to 7 μg/kg is presented in Fig. 1.

Plasma calcium. Heparinized blood was obtained from the Japanese quail by heart puncture following light anesthesia with halothane (Fluothane, Ayerst Laboratories, Inc., New York, N.Y.) and prior to removal of the kidneys. Plasma calcium concentration was determined by atomic absorption spectrophotometry (Perkin–Elmer

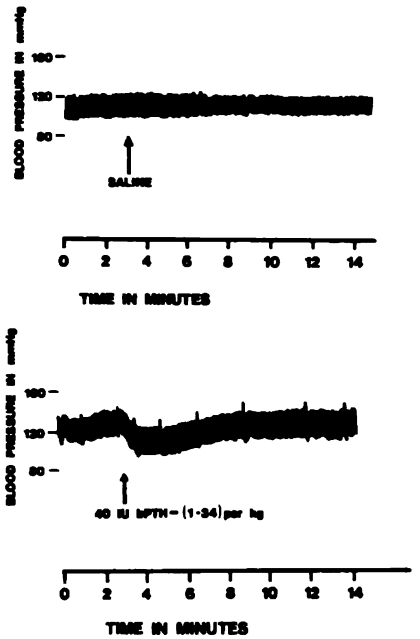

FIG. 1. Typical hypotensive response to bPTH(1-34) in the anesthetized rat. Upper panel: control injection of saline. Lower panel: response to 40 IU/kg of bPTH(1-34).

Model 303) following dilution with 0.5% lanthanum chloride and deproteinization with 5% trichloroacetic acid.

Results. Both unoxidized bPTH(1-84) and unoxidized bPTH(1-34) significantly enhanced renal 1-hydroxylase activity and suppressed 24-hydroxylase activity. Mild oxidation with hydrogen peroxide did not significantly modify these responses (Table I). The hypercalcemic activity of bPTH(1-34) was unaffected by oxidation, whereas that of bPTH(1-84) was markedly reduced (Table II). In contrast, oxidation essentially eliminated the hypotensive (Fig. 2), and significantly reduced the renal adenylate cyclase (Table III), activities of bPTH(1-34). The plasma calcium concentrations were normal 12 hr following injection in all birds as anticipated; the hypercalcemic response to bPTH is very rapid in onset and short in duration in Japanese quail (15).

Discussion. The significance of this work becomes more apparent by placing it within the context of our other structure–activity studies associated with hydrogen peroxide treatment of bPTH(1-34). Whereas the hypercalcemic activity of intact bPTH(1-84) is largely inactivated by mild oxidation with hydrogen peroxide, that of the synthetic fragment, bPTH(1-34), is unaffected by such treatment (3, 4). Prior to our report, apparently no laboratory had investigated the *hypercalcemic* activity of oxidized synthetic bPTH(1-34). Mild oxidation is assumed to affect only the methionine residues at positions 8 and 18 which occur in both bPTH(1-34) and bPTH(1-84). Why, then, does such treatment affect the activity of one molecule and not the other? Perhaps

TABLE I. EFFECT OF H_2O_2 ON THE RESPONSE OF THE RENAL 1- AND 24-HYDROXYLASES TO bPTH(1-34) AND bPTH(1-84) IN 4-WEEK-OLD MALE JAPANESE QUAIL

Treatment	Dose, im (per bird)	No. birds	Plasma Ca (mg/dl)	Metabolite production (pmole·min^{-1}·g^{-1} kidney)	
				$1,25\text{-}(OH)_2D_3$	$24,25\text{-}(OH)_2D_3$
Control, acid saline	0.4 ml	5	9.8 ± 0.24	2.4 ± 2.35	27.6 ± 3.44
bPTH(1-34)	20 μg	5	9.6 ± 0.16	17.2 ± 1.82**	12.7 ± 3.37*
bPTH(1-34) + H_2O_2	20 μg	5	9.4 ± 0.21	22.5 ± 3.57**	6.4 ± 4.03**
bPTH(1-84)	17 μg	5	9.5 ± 0.25	8.3 ± 3.24***	12.4 ± 4.25*
bPTH(1-84) + H_2O_2	17 μg	5	9.5 ± 0.26	10.9 ± 1.98*	8.0 ± 3.30**

Note. Results are means ± SE. bPTH(1-34): synthetic bovine PTH(1-34) from Beckman (6000 IU/mg claimed potency). bPTH(1-84): purified bPTH(1-84) from National Institute for Biological Standards and Control, London (NIBSC 77/533) with an assigned potency of 2300 IU/mg.

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.10$ (one-tail test).

TABLE II. EFFECT OF H_2O_2 ON THE HYPERCALCEMIC RESPONSE TO bPTH(1-34) AND bPTH(1-84) IN JAPANESE QUAIL

Treatment	Dose, iv (per bird)	No. birds	Plasma Ca (mg/dl, mean ± SE)
Assay P120			
Control, vehicle alone	0.4 ml	7	10.1 ± 0.28
bPTH(1-34)	7 μg	7	12.2 ± 0.34***
H_2O_2-treated bPTH(1-34)	7 μg	7	13.0 ± 0.16***
Assay P162[a]			
Control, vehicle alone	0.4 ml	6	9.4 ± 0.21
bPTH(1-84)	44 μg	5	12.4 ± 0.31***
H_2O_2-treated bPTH(1-84)	44 μg	6	10.2 ± 0.52

Note. See footnotes to Table I. Vehicle: 51 m*M* $CaCl_2$ + 0.01% bovine serum albumin.
[a] These data (assay P162) are reproduced from Pang *et al.* (4).

oxidation of the two methionines at positions 8 and 18, respectively, results in conformational changes such that the intact bPTH(1-84) molecule, with its large biologically inactive C-terminal tail, cannot adequately interact with its receptor. The synthetic N-terminal fragment, bPTH(1-34), on the other hand, may not experience any steric hindrance resulting from such a conformational change.

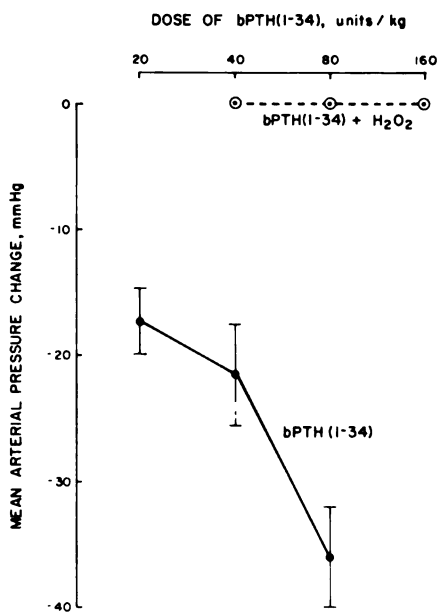

FIG. 2. Effect of different doses of untreated and oxidized bPTH(1-34) on mean arterial pressure in the anesthetized rat.

Equally important is our finding that mild oxidation partially or possibly totally inactivates some but not all of the responses associated with the synthetic bPTH(1-34) fragment. Of the responses studied to date, three resist inactivation. These are: (i) the hypercalcemic response in the Japanese quail (3, 4); (ii) the hypocalciuric response in the rat (16); and now, from the present study, (iii) the ability to activate the renal 25-hydroxyvitamin D_3-1-hydroxylase and suppress the 25-hydroxyvitamin D_3-24-hydroxylase enzymes in the Japanese quail. All three responses are classically associated with the mobilization of calcium into extracellular fluid from bone, kidney, and gut. Other responses studied are partially or totally eliminated by oxidation of bPTH(1-34). These include: (i) the hyperphosphaturic response in the rat (5); (ii) renal adenylate cyclase activation in the Japanese quail (Table III); (iii) relaxation of induced contraction of smooth muscles (6); and (iv) the hypotensive response in the rat ((4) and Fig. 2).

One other interesting point emerges from these findings. It is obvious that the mechanisms involved in the hypercalcemic, hypocalciuric, and renal 1-hydroxylase responses demand structural requirements in the bPTH(1-34) molecule which are differ-

TABLE III. EFFECT OF H_2O_2 ON THE AVIAN RENAL ADENYLATE CYCLASE RESPONSE TO bPTH(1-34)

Treatment	Dose (per tube)	Adenylate cyclase activity (pmole cAMP/mg protein)
Control	—	101 (87–115)
bPTH(1-34)	0.1 μg	408 (361–454)
bPTH(1-34) + H_2O_2	0.1 μg	131 (121–141)

Note. Results are means; range of duplicates in parentheses. bPTH(1-34): synthetic bPTH(1-34) from Peninsula (10,000 IU/mg).

ent from those needed to effect the responses, such as the hyperphosphaturic, renal adenylate cyclase, hypotensive, and smooth muscle responses, which are not elicited by the oxidized form of bPTH(1-34). Is it possible that the different structural requirements revealed in our functional studies using the oxidation technique approach are related to the existence of different types of receptors? Further work is needed before this question can be answered with assurance.

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1. Tashjian AH, Ontjes DA, Munson PL. Alkylation and oxidation of methionine in bovine parathyroid hormone: Effects on hormonal activity and antigenicity. Biochemistry 3:1175–1182, 1964.
2. Dacke CD, Kenny AD. Avian bioassay method for parathyroid hormone. Endocrinology 92: 463–470, 1973.
3. Kenny AD, Pang PKT. Failure of bPTH(1-34) to be inactivated by oxidation with hydrogen peroxide. Calcif Tissue Int 31:73, 1980.
4. Pang PKT, Yang MCM, Keutmann HT, Kenny AD. Structure activity relationship of parathyroid hormone: separation of the hypotensive and the hypercalcemic properties. Endocrinology, in press.
5. Kenny AD, Pang PKT. Dissociation of the hypercalcemic and hyperphosphaturic actions of bovine parathyroid hormone (1-34). Fed Proc 40:399, 1981.
6. Shew RL, Yee JA, Pang PKT, Kenny AD. Direct effect of parathyroid hormone on rat uterine contraction. Anat Rec 199:234A, 1981.
7. Baksi SN, Kenny AD. Vitamin D_3 metabolism in immature Japanese quail: Effects of ovarian hormones. Endocrinology 101:1216–1220, 1977.
8. Kenny AD. Vitamin D metabolism: Physiological regulation in egg-laying Japanese quail. Amer J Physiol 230:1609–1615, 1976.
9. Baksi SN, Kenny AD. Effects of administration of antiestrogen (tamoxifen) *in vivo* on the metabolism of 25-hydroxyvitamin D_3 *in vitro* in the Japanese quail. Biochem Pharmacol 26:2439–2443, 1977.
10. Baksi SN, Kenny AD. Vitamin D metabolism in aged Japanese quail: Dietary calcium and estrogen effects. Amer J Physiol 241:E275–E280, 1981.
11. Marcus R, Aurbach GD. Bioassay of parathyroid hormone *in vitro* with a stable preparation of adenyl cyclase from rat kidney. Endocrinology 85:801–810, 1969.
12. White AA, Zenser TV. Separation of cyclic 3',5'-nucleoside monophosphates from other nucleotides on aluminum oxide columns. Application to the assay of adenyl cyclase and guanyl cyclase. Anal Biochem 41:372–396, 1971.
13. Forte LR, Nickols GA, Anast CA. Renal adenylate cyclase and the interrelationship between parathyroid hormone and vitamin D in the regulation of urinary phosphate and adenosine cyclic 3',5'-monophosphate excretion. J Clin Invest 57:559–568, 1976.
14. Pang PKT, Tenner Jr TE, Yee JA, Yang M, Janssen HF. Hypotensive action of parathyroid hormone preparations on rats and dogs. Proc Nat Acad Sci USA 77:675–678, 1980.
15. Kenny AD, Dacke CD. The hypercalcaemic response to parathyroid hormone in Japanese quail. J Endocrinol 62:51–53, 1974.
16. Kenny AD, Pang PKT. Effect of oxidation on the urinary calcium response in rats to bovine parathyroid hormone. Calcif Tissue Int, in press.

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TABLE I. BINDING AND DISPLACEMENT OF α- AND α_2-ADRENERGIC RADIOLIGANDS IN RAT PANCREATIC ISLET CELLS

Ligand	B_{max} (fmol/mg protein)	K_d (nM)	K_i (μM) Displacement by			
			Epinephrine (n = 6)	Norepinephrine (n = 6)	Isoproterenol (n = 6)	Clonidine (n = 6)
α_2-Clonidine (5)	50.4 ± 3.6[a]	0.552 ± 0.09	0.150 ± 0.008	0.420 ± 0.013	16.0 ± 1.7	0.523 ± 0.10 nM
α-Dihydroergocryptine (5)	55.0 ± 1.1	0.325 ± 0.11	0.183 ± 0.016	0.183 ± 0.016	9.9 ± 2.9	0.488 ± 0.09 nM

[a] Results are means + SEM. Determination of B_{max} and K_d was done on five animals. K_i on six animals. There is no significant difference between epinephrine and norepinephrine in their ability to displace the α and α_2 radioligands. Both radioligands are displaced most effectively by clonidine.

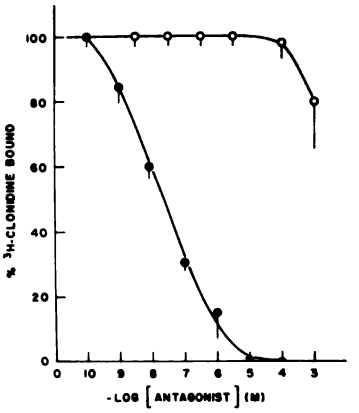

FIG. 3. Displacement curves for yohimbine (●) and prazosin (○) vs 10 nM [^{3}H]clonidine; yohimbine (α_2-antagonist) was found to displace the bound clonidine with an affinity (K_i) similar to that for displacing [^{3}H]dihydroergocryptine. Prazosin (α_1-antagonist) was ineffective suggesting that α-receptors on the pancreatic islet cells are of the α_2 type.

Fig. 4. The K_i for yohimbine was 7.1 ± 75 nM, again indicating the preferential displacement at the α_2-adrenergic receptor

The use of adrenergic receptor agonists and antagonists *in vivo* and *in vitro* has led to the generally accepted working hypothesis that α-adrenergic receptors on

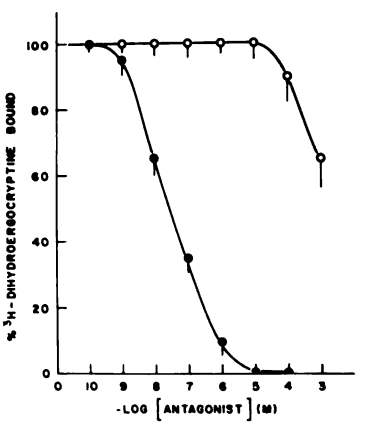

FIG. 4. Displacement curves for yohimbine (●) and prazosin (○) vs 10 nM [^{3}H]dihydroergocryptine. Yohimbine displaced the bound radioligand from a single population of α-receptors whereas prazosin was ineffective. The similarity of the data for the two radioligands (also in Table I) indicates that [^{3}H]dihydroergocryptine binds to α_2-receptors in the islets.

pancreatic β cells inhibit insulin secretion whereas the β-adrenergic receptors stimulate it. With the availability of more specific adrenergic agonists and antagonists it has been suggested that the effects on insulin secreting responses are exerted by discrete subtypes of receptor population. Thus clonidine, which is an α_2-receptor agonist, has been shown to inhibit insulin secretion (7) and this response is blocked most effectively by the α_2-antagonists, e.g., yohimbine, and not by α_1-antagonists, e.g., prazosin (8).

The present study provides more direct measurements of adrenergic receptor density and affinity by using radioligands. Clearly, the data on both the displacement of [^{3}H]clonidine by the various agonists (Fig. 2) and by the two specific antagonists (Fig. 3) support the conclusion that the α-adrenergic receptors on the rat pancreatic islets are of the α_2 subtype.

The mechanisms whereby the various types of adrenergic receptors exert their effect on insulin secretion are unknown. Some suggestions have been made regarding their role in adipose tissues metabolism (9). In this tissue activation of β-adrenergic receptors stimulates adenylate cyclase activity and activation of α_2-receptors is believed to counter this stimulation. The general applicability of these mechanisms remains uncertain inasmuch as α_2-receptors have been reported only in human and hamster fat cells (10) but not in rat adipocytes (11).

Attempts to implicate cyclic AMP in the α-adrenergic inhibition of insulin secretion in rat pancreatic islets have given contradictory results. Although addition of clonidine to such cells clearly inhibited glucose-induced insulin secretion, there was no affect on adenylate cyclase activity (12). By contrast, it has also been reported that clonidine, epinephrine, and norepinephrine were effective in diminishing the glucose-induced accumulation of cyclic AMP in islet cells (8). The reason for the discrepant data is not clear.

It is of interest that glucose per se increases islet adenylate cyclase activity and results in accumulation of cyclic AMP in the islets. Likewise, stimulation of β-adrenergic receptors increases the adenylate cyclase and accumulation of cyclic AMP. Although the β-adrenergic receptor blocker, propranolol, decreases insulin secretion evoked by a glucose load (13) it is not known if there is a link between β-adrenergic receptors and insulin secretion evoked by glucose or whether the β-adrenergic system participates at some later stage in insulin secretion. Also it is conceivable that propranolol inhibited insulin secretion through its local anesthetic effect which is independent of its adrenergic blocking action.

Last, it has been reported, in abstract form, that addition of epinephrine to islets increased insulin secretion when glucose was absent from the media, but it decreased insulin secretion in the presence of glucose (14). We have found that presence or absence of glucose in the media has no effect on the α- or β-adrenergic receptor density or affinity (1, 2). Thus it appears likely that the factors which determine whether the adrenergic influence will increase or decrease insulin secretion are at sites beyond the adrenergic receptors.

1. Cherksey B, Altszuler N, Zadunaisky, J. Preponderance of β-adrenergic binding sites in pancreatic islet cells of the rat. Diabetes 30:172–174, 1981.
2. Cherksey B, Mendelsohn S, Zadunaisky J, Altszuler N. Displacement of α- and β-radioligands by specific adrenergic agonists in rat pancreatic islets. Submitted for publication, 1982.
3. Lacy PE, Kostianovsky MM. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16:35–39, 1967.
4. Cherksey B, Zadunaisky J, Murphy RB. Cytoskeletal constraint of the β-adrenergic receptor in frog erythrocyte membranes. Proc Nat Acad Sci USA 77:6401–6405, 1980.
5. Scatchard G. The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51:660–672, 1949.
6. Cheng Y, Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibition which causes 50 percent inhibition (I_{50}) of an enzyme reaction. Biochem. Pharmacol. 22:3099–3108, 1973.
7. Metz SA, Halter JB, Robertson RP. Induction of defective insulin secretion and impaired glucose

tolerance by clonidine. Diabetes 27:554–562, 1978.

8. Yamazaki S, Katada T, Ui M. Alpha$_2$-adrenergic inhibition of insulin secretion via interference with cyclic AMP generation in rat pancreatic islets. Mol Pharmacol 21:648–653, 1982.
9. Garcia-Sainz JA, Fain JN. Regulation of adipose tissue metabolism by catecholamines: Roles of alpha$_1$, alpha$_2$, and beta-adrenoreceptors. Trends Pharmacol Sci (TIPS) 3:201–203, 1982.
10. Burns TW, Langley PE, Terry BE, Bylund DB, Hoffman BB, Tharp MD, Lefkowitz RJ, Garcia-Sainz JA, Fain JN. Pharmacological characterization of adrenergic receptors in human adipocytes. J Clin Invest 67:467–475, 1981.
11. Fain JN, Garcia-Sainz JA. Role of phosphatidylinositol turnover in alpha$_1$ and of adenylate cyclase inhibition in alpha$_2$ effects of catecholamines. Life Sci 26:1183–1194, 1980.
12. Leclercq-Meyer V, Herchuelz A, Valverde I, Couturier E, Marchand J, Malaisse WJ. Mode of action of clonidine upon islet function. Dissociated effects upon the time course and magnitude of insulin release. Diabetes 29:193–200, 1980.
13. Cerasi E, Luft R, Efendic S. Effect of adrenergic blocking agents on insulin response to glucose infusion in man. Acta Endocrinol 69:335–346, 1972.
14. Wollheim CB, Sharp GWG. Stimulatory and inhibitory effects of epinephrine on islet Ca^{++} uptake and insulin release. Diabetologia 15:282, 1982 (Abstract).

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Myoelectric Activity of the Diverted Antroduodenum in the Dog[1] (41499)

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Abstract. Electrodes were chronically implanted on the gastric antrum and the orad duodenum of four dogs. After implantation, fasted and fed state myoelectric activity was monitored. After control testing, the stomach was bisected at the orad antral margin and the antrum was closed. Bowel continuity was restored with gastrojejunostomy. Extrinsic nerves of the antroduodenal segment were maintained. After surgery, fasted and fed state myoelectric activity were reassessed. Surgical diversion of the antroduodenum was previously shown to result in chronic hypergastrinemia of a postprandial magnitude. It also resulted in separation of the antrum from its myoelectric pacemaker in the orad corpus, and a chronic absence of digesta from the diverted segment. In the present experiments both the antrum and duodenum exhibited unchanged migrating myoelectric complex periods. In addition, the duodenal BER frequency was unchanged. In contrast, the antral BER frequency was reduced by half. The percentage of antral and duodenal BER superimposed with spike potentials in response to liquid or solid meals was unchanged postoperatively despite the absence of food in the diverted segment, and despite the absence of postprandially elevated gastrin. We conclude that a hormone other than gastrin, and/or a neurally conducted impulse generated by the physical presence of the food in the gastric remnant, contributed to the postprandial generation of spike potentials in the diverted antroduodenum.

Distinct electric and motor patterns have been characterized in dogs in both the fasted and fed states (1, 2). Meals of liquid and solid foods elicit a characteristic antroduodenal myoelectric pattern where one or two duodenal spike potentials immediately follow the antral BER and spike potential (3, 4). This well-timed spike potential activity corresponds to antral and duodenal contractions that coordinate gastric emptying. Emptying probably occurs as a result of an antral contraction pushing chyme into a relaxed duodenum, immediately followed by duodenal contractions that propel the gastric effluent caudally (5, 6). Because this electric pattern exists postprandially only, the physical presence of food seems prerequisite to pattern development (3).

We have recently reported the use of an experimental model where the stomach was bisected, the orad antrum closed, and tract continuity restored via gastrojejunostomy (antroduodenal exclusion) (7). This procedure resulted in chronic diversion of ingesta from the antrum and duodenum, and a chronic hypergastrinemia which was not further elevated by feeding. The postprandial elevation of gastrin has long been postulated to play a major role in generating gastric (8, 9) and intestinal (10) spike potentials and has tacitly been assigned a role in the generation of postprandial electric patterns. In this study we tested whether the diverted antroduodenum could still exhibit the usual postprandial electric pattern, despite no direct contact with food, and an absence of postprandially elevated gastrin. We report here that the diverted antroduodenum did exhibit a significant, though reduced, amount of coordinated electric activity postprandially. We suggest that an extrinsic neural reflex and/or a nongastrin hormonal effect may have initiated this activity.

Materials and Methods. *Surgical model.* The surgical preparation of this model in our laboratory has recently been de-

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scribed (7). Briefly, four dogs of mixed breed (10–20 kg) were anesthetized with pentobarbital, 30 mg/kg intravenously (Abbott Lab). Silver monopolar electrodes whose construction and method of implantation have been reported (4) were implanted on the gut. One electrode was sutured to the serosa of the gastric antrum ca. 2–4 cm orad to the gastroduodenal junction, and two electrodes were implanted on the duodenum ca. 2 and 4 cm caudad to the gastroduodenal junction. A reference electrode was sewn into the subcutaneous tissue of the left flank. A 2-week recovery period was allowed after which control testing was performed. After testing, the animals were again prepared for surgery. At celiotomy, the stomach and attending neurovascular processes were transected at the gastric incisura. Care was taken to preserve the nerve and vascular supply to the antrum. The antral margin was then closed, removing the gastric antrum from its electric pacemaker in the orad corpus. The gastric remnant was partially closed and anastomosed end-to-side to the jejunum ca. 10 cm caudad to the ligament of Treitz causing antroduodenal exclusion from the normal digestive path (Fig. 1). After a two-week recovery the dogs were retested. In all cases an 18-hr fast preceded the tests.

Tests. Several myoelectric parameters in the interdigestive and digestive states were compared pre- and postoperatively to characterize the motility of the diverted antroduodenal segment.

Interdigestive activity. Antral and duodenal interdigestive migrating myoelectric complexes (MMC) were identified. The interdigestive state is marked by a continuum of myoelectric activity phases where phases 1, 2, and 3 represent intervals of low, intermediate, and high numbers of spike potentials, respectively, superimposed upon the basic electric rhythm (BER) of the stomach and small bowel (1). The periodicity of the MMC (time interval from phase 3 to the subsequent phase 3, usually ca. 100 min) was measured for the antrum and duodenum and each were compared pre- and postoperatively. Both antral and duodenal BER frequencies were calculated

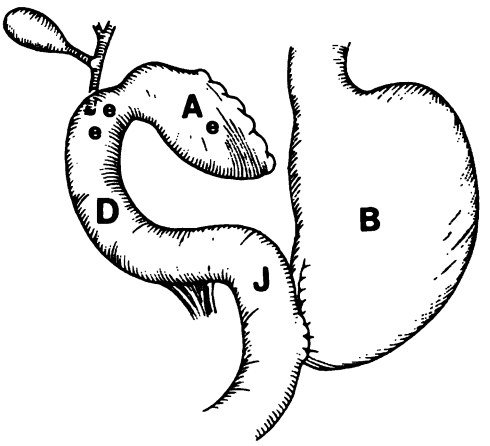

FIG. 1. Surgical diversion of the gastric antrum and duodenum. (A) Antrum, (B) gastric body, (D) duodenum, (J) jejunum, (e) electrode.

from samples taken from the first 30 min of phase 1. Postoperative interdigestive antral records were further examined qualitatively for the presence of antral BER cycles that were of greater than normal frequency (>5 BER/min), or for the presence of duodenal BER superimposed upon the antral BER.

Digestive state activity. Antral and duodenal spike potential responses to both liquid and solid meals were each compared pre- and postoperatively. The composition and gastric emptying properties of the liquid meal have been described (11). At the beginning of phase 1 of the interdigestive state, 300 ml of a citrate–fat liquid test meal was fed via an oral-gastric tube. Test meals were administered during phase 1 to prevent confounding of the postprandial myoelectric response by spontaneous (i.e., phase 2 or 3) spike potential activity. The percentage of antral and duodenal BER superimposed with spike potentials in response to the meal was determined for the 30 min immediately following feeding. This method was also used to determine the response to 250 g of canned dog food (Vets, Perk Foods Co. Inc.). However, after feeding of solid food, the response was quantified from 15 to 45 min.

In addition to the presence of duodenal spike potentials, their distribution relative

to the antral BER cycle was examined. In unoperated dogs, postprandial spike potentials are superimposed on the first and/or second duodenal BER that immediately follows the antral BER (3). Because the ratio of duodenal to antral cycles is ca. 4.5:1, the duodenal spike potentials usually appear concurrently with the first half of the antral cycle. This electric relationship was considered to be retained postoperatively if the duodenal spike potentials occurred only during the first half of the antral cycle. The percentage of antral cycles so accompanied by duodenal spike potentials was determined pre- and postoperatively after both liquid and solid meals.

Data analysis. Control and postoperative values obtained during the digestive and interdigestive states were each compared using the *t* test for unpaired values. In all cases, values used in the comparisons represent the mean of one to three observations per dog.

Results. *Interdigestive state.* Surgical separation of the gastric antrum from its pacemaker in the orad corpus resulted in a reduced antral BER frequency (bradygastria, Table I), and the cycles were arrhythmic (Fig. 2, A vs B and C). In contrast, the duodenal BER frequency was unaltered postoperatively (Table I). There was no evidence that the duodenal pacemaker hastened the frequency of the unpaced, slowed antral BER, and there was no superimposition of duodenal rhythms upon the antral record. Neither the diverted antrum nor the duodenum exhibited changes in the MMC period postoperatively (Table I).

Digestive state. Feeding abolished migrating myoelectric complexes on the diverted segment. Both the antrum and the duodenum exhibited fed state electric patterns after meals of liquid and solid despite being removed from the digestive path.

Preoperatively, the usual temporal relationship between postprandial duodenal spike potentials and the antral BER cycle, as first described by Allen *et al.* (3) was confirmed. In contrast, after antroduodenal exclusion, postprandial duodenal spike potentials either accompanied the first half of the arrhythmic antral cycle (Fig. 2B), or occurred throughout the duration of the antral cycle (Fig. 2C). These two postoperative patterns appeared with equal frequency after both liquid and solid meals.

Antroduodenal exclusion did not affect the overall percentage of antral or duodenal BER which were associated with spike potentials in response to liquid and solid meals (Table II). However, the distribution of postprandial duodenal spike potentials was changed postoperatively. Preoperatively, after solid food, 80 ± 6% of the antral cycles were accompanied by duodenal spike potentials which appeared on the first and/or second BER immediately following the antral cycle (Fig. 2A). Postoperatively, after solid food, 41 ± 6% of the antral cycles remained temporally associated with duodenal spike potentials. In contrast, after the liquid meal, the degree of temporally related response was not changed post-

TABLE I. INTERDIGESTIVE BASIC ELECTRIC RHYTHM (BER) FREQUENCIES AND MIGRATING MYOELECTRIC COMPLEX (MMC) PERIODS BEFORE AND AFTER ANTRODUODENAL EXCLUSION

Parameter	Organ	Values		P^a
		Preop	Postop	
BER frequency (cpm)	Antrum	5.1 ± 0.1[b] (3)[c]	2.2 ± 0.4 (3)	<0.005
	Duodenum	18.8 ± 0.7 (3)	18.2 ± 0.3 (3)	>0.05
MMC period (min)	Antrum	110 ± 18 (4)	105 ± 9 (3)	>0.05
	Duodenum	111 ± 7 (4)	97 ± 6 (3)	>0.05

[a] *t* test for unpaired values.
[b] Mean ± SEM.
[c] Number of dogs.

pus, without damaging the gastroduodenal junction. Postoperative bradygastric signals remained independent of the duodenal pacemaker influence since no evidence of antral tachygastria (>5 cpm) was obtained. This confirms the presence of a functional insulatory zone and supports the conclusion (19) that transpyloric BER conduction does not influence antral or duodenal BER frequencies.

1. Code CF, Marlett JA. The interdigestive myoelectric complex of the stomach and small bowel of dogs. J Physiol 246:289–309, 1975.
2. Carlson GM, Ruddon RW, Hug CC Jr, Bass P. Effects of nicotine on gastric antral and duodenal contractile activity in the dog. J Pharmacol Exp Ther 172:367–376, 1970.
3. Allen GL, Poole EW, Code CF. Relationships between electrical activities of antrum and duodenum. Amer J Physiol 207:906–910, 1964.
4. McCoy EJ, Bass P. Chronic electrical activity of gastroduodenal area: Effects of food and certain catecholamines. Amer J Physiol 205:439–445, 1963.
5. Carlson HC, Code CF, Nelson RA. Motor action of the canine gastroduodenal junction: A cineradiographic, pressure, and electric study. Amer J Dig Dis 11:155–172, 1966.
6. Bass P, Russell J. Gastric emptying of liquids: Role of the small intestine. In: Chey WY, ed. Functional Disorders of the Digestive Tract. Raven Press, New York, in press, 1983.
7. Russell J, Bass P, Shimizu M, Miyauchi A, Go VLW. Canine intestinal ulcer: Myoelectric components and the effect of chronic hypergastrinemia. Gastroenterology 82:746–752, 1982.
8. Kelly KA. Effect of gastrin on gastric myoelectric activity. Amer J Dig Dis 15:399–405, 1970.
9. Thomas PA, Schang J, Kelly KA, Go VLW. Can endogenous gastrin inhibit canine interdigestive gastric motility? Gastroenterology 78:716–721, 1980.
10. Weisbrodt NW, Copeland EM, Kearley RW, Moore EP, Johnson LR. Effects of pentagastrin on electrical activity of small intestine of the dog. Amer J Physiol 227:425–429, 1974.
11. Weisbrodt NW, Wiley JN, Overholt BF, Bass P. A relation between gastroduodenal muscle contractions and gastric emptying. Gut 10:543–548, 1969.
12. Thomas PA, Kelly KA. Hormonal control of interdigestive motor cycles of canine proximal stomach. Amer J Physiol 237:E192–E197, 1979.
13. Eeckhout C, De Wever I, Peeters T, Hellemans J, Vantrappen G. Role of gastrin and insulin in postprandial disruption of migrating complex in dogs. Amer J Physiol 235:E666–E669, 1978.
14. Bueno L, Ruckebusch M. Insulin and jejunal electrical activity in dogs and sheep. Amer J Physiol 230:1538–1544, 1976.
15. Mukhopadhyay AK, Thor PJ, Copeland EM, Johnson LR, Weisbrodt NW. Effect of cholecystokinin on myoelectric activity of small bowel of the dog. Amer J Physiol 232:E44–E47, 1977.
16. Mukhopadhyay AK, Johnson LR, Copeland EM, Weisbrodt NW. Effect of secretin on electrical activity of small intestine. Amer J Physiol 229:484–488, 1975.
17. Engstrom ER Jr, Webster JG, Bass P. Analysis of duodenal contractility in the unanesthetized dog. IEEE Trans Biomed Eng BME-26:517–523, 1978.
18. Bass P, Code CF, Lambert EH. Electric activity of gastroduodenal junction. Amer J Physiol 201:587–592, 1961.
19. Gladen HE, Kelly KA. Independence of canine gastric and duodenal pacesetter potentials shown by electric pacing. Mayo Clinic Proc 52:51–53, 1977.
20. Gullikson GW, Okuda H, Shimizu M, Bass P. Electrical arrhythmias in gastric antrum of the dog. Amer J Physiol 239:G59–G68, 1980.

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Ovulation, Ovarian 17α-Hydroxylase Activity, and Serum Concentrations of Luteinizing Hormone, Estradiol, and Progesterone in Immature Rats with Diabetes Mellitus Induced by Streptozotocin (41500)

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Abstract. Immature female rats were injected with streptozotocin (60 mg/kg, iv) 3 to 4 days prior to the injection (iv) of 20 IU of pregnant mare's serum gonadotropin (PMS). Animals were killed at various intervals and the serum levels of estradiol, luteinizing hormone (LH), and progesterone were determined by radioimmunoassays. The ovarian steroid 17α-hydroxylase activity was determined by a tritium exchange assay using pregnenolone as the substrate. Ovulation was determined 72 hr after PMS by flushing of the oviducts. The diabetes mellitus induced by the drug reduced the number of animals ovulating and in some animals the number of ova shed when compared to controls. However, a surge in LH, which reached a peak at 60 hr, was seen in the diabetic animals; a larger peak with the same timing was found in the controls. Changes in ovarian 17α-hydroxylase also indicated that an increase in LH release occurred in the diabetic animals at about 60 hr. Estradiol levels were higher, but progesterone levels lower, in diabetic than control animals. Administration of 1 mg of progesterone to diabetic animals 48 hr after PMS resulted in an increase in the number of animals ovulating and the number of ova shed. The results indicate that hyperglycemia induced by streptozotocin is not inconsistent with production of an LH surge or with ovulation following ovarian stimulation by PMS. However, the lowered production of progesterone, which may be a cause or a result of lowered LH output, appears to be a primary factor in the reduced ovulatory rate.

Female infertility is a well-known consequence of diabetes mellitus regardless of whether the syndrome was induced by pancreatectomy or by pharmacologic agents (1). A consistent finding, and obviously an important factor, in the infertility is a reduction in the percentage of animals ovulating and the number of eggs ovulated per animal (2, 3). In recent studies (4, 5) immature female rats, made diabetic with alloxan, failed to ovulate subsequent to stimulation of the ovary by pregnant mare's serum gonadotropin (PMS), even though follicular maturation and estrogen production were not different from that in control animals. Several lines of evidence indicated that the site of the primary lesion was the hypothalamic–hypophyseal axis. Specifically, the lack of an LH surge, secondary to a reduced pituitary response to hypothalamic LH releasing hormone, was interpreted as the diabetes-induced defect (5). The present study was undertaken to examine the ovarian steroidogenic and ovulatory responses, as well as changes in serum levels of LH, in animals made diabetic with the antibiotic streptozotocin. The latter drug is less toxic than alloxan, but very effective at removing pancreatic beta cell activity (6).

The results demonstrate that treatment with streptozotocin causes a typical syndrome of diabetes mellitus and a reduction in the ovulatory LH surge in immature rats injected with PMS. The number of animals ovulating and the number of ova shed are reduced in these diabetic animals but both indices of ovulatory function can be restored to normal by the administration of progesterone 48 hr after the PMS.

Materials and Methods. Immature (26–30 day) female rats of the Holtzman strain were housed 5–10 animals/cage in

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temperature (25 ± 2°) and light (12 hr light/day) controlled quarters and given free access to food and water. Diabetes mellitus was induced by the intravenous injection (iv) of streptozotocin (Sigma Chemical Co., St. Louis, Mo.). The drug was dissolved immediately before use in 0.01 *M* citrate buffer (pH 4.5) and used at a dose of 60 mg/kg body weight; controls received only the buffer. The animals which received the drug demonstrated polydipsia and polyphagia within 48 hr. Hyperglycemia was verified at the time of sacrifice by determination of the concentration of serum glucose using the glucose oxidase method, with kits obtained from Sigma. Animals having serum glucose concentration levels more than twice that of controls were considered diabetic.

Three to four days after administration of streptozotocin, 20 IU of PMS (Sigma), dissolved in 0.15 *M* NaCl, was injected (iv) while the animals were under light ether anesthesia. The animals were decapitated at various times after injection of the PMS and the blood from the trunk collected in 12 × 75-mm glass tubes. After clotting at room temperature, the blood was centrifuged at 2000*g* for 20 min and the serum stored at −20° until assayed for hormone content.

The ovaries were removed as quickly as possible after decapitation, cleaned of adhering tissue and weighed to the nearest 0.1 mg on a torsion balance. When 17α-hydroxylase activity was to be measured the ovaries were quickly frozen, and stored, at −20°. In animals killed at 72 hr after PMS the oviducts were flushed with 0.15 *M* NaCl containing hyaluronidase into a depression slide and the number of ova counted with the aid of a dissecting microscope.

Serum LH concentrations were determined by double antibody radioimmunoassays using kits supplied by NIAMDD-Rat pituitary program: details of the procedure have been reported previously (7). Anti-rat LH antiserum No. 3, with an initial dilution of 1:10,000, was used with rat LH-RP-1 as the standard. Hormones were iodinated by a modification of the Butt method (8).

Serum levels of estradiol and progesterone were determined by radioimmunoassay. Tritiated estradiol, (2,4,6,7-^{3}H(N)estradiol-17β; 115 Ci/mmole) and progesterone (1,2,6,7-^{3}(N)progesterone; 97.9 Ci/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.) and used without further purification. Anti-estradiol antiserum was obtained from Dr. D. Exley (University of Liverpool, England) (9) and anti-progesterone antiserum was prepared by Dr. Vernon Stevens (Ohio State University, Columbus). Details of the assay procedure have been published (10).

Ovarian 17α-hydroxylase activity was measured by a tritium exchange assay using pregnenolone as the substrate (11). The ovaries were homogenized in 0.15 *M* KCl and centrifuged at 10,000*g* for 30 min. The supernatant solution was centrifuged at 105,000*g* for 60 min and the pellet (microsomal fraction) was then resuspended in 0.15 *M* Na-K PO_4 buffer (pH 7.4). At least triplicate samples of this suspension were assayed; results were expressed as nanomoles pregnenolone converted per hour per milligram of microsomal protein.

Data were subjected to analysis of variance or χ^2 analyses. The independent Student's *t* test was applied when appropriate. Differences between means with a *P* value less than 0.05 were considered statistically significant.

Results. In two large groups of rats, the 60 mg/kg dose of streptozotocin produced a mean nonfasting serum glucose concentration of 410 ± 27 mg/100 ml (n = 126) compared to 119 ± 3.2 mg/100 ml (n = 116) in the controls. While 24 of the 26 control animals killed at 72 hr after injection of PMS had ovulated an average of 12.6 ± 3.2 ova, only 6 of the 26 diabetic animals had ovulated 11.2 ± 1.2 ova.

The ovarian weight responses to 20 IU of PMS were not significantly different between controls and diabetic animals (Fig. 1). However, the steroidogenic responses did shown differences. In Fig. 2 the changes in serum estradiol levels are shown for groups of 10 rats (experiment A) killed at various times after injection of PMS. The diabetic animals had the same level of es-

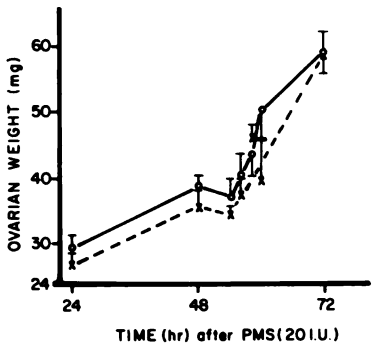

FIG. 1. Ovarian weight increases in control (O) and diabetic (x) immature rats of experiments A and B, injected (iv) at time 0 with 20 IU of PMS. Diabetes was induced by injection (iv) of 60 mg/kg of streptozotocin 3–4 days before the PMS. Vertical lines represent one SEM for groups of 16 rats.

tradiol 24 hr after PMS but by 48 hr it was 32% higher than that in controls ($P < 0.01$). The difference in concentration between control and diabetic animals was not statistically significant at 54 hr, but because of the rapidly falling values which occurred only in controls after 54 hr, differences at 56 and 58 hr were pronounced. The estradiol levels at 60 hr were 95% higher in the diabetic animals but by 72 hr they were the same in the two kinds of animals.

The experiment was repeated one month later using 6 animals per group (experiment B) with quantitatively slightly different results, even though the same lot of PMS was used in both cases. Ovarian weight increases were not different from those found in animals of experiment A and are included with them in the data of Fig. 1. The pattern of changes was the same as in rats of experiment A but higher levels of estradiol were found with the animals of experiment B (Fig. 3). As with experiment A diabetic animals had higher levels of serum estradiol than did controls, with the largest differences occurring between 54 and 60 hr after PMS. While the serum estradiol levels in controls in the two experiments were not different, the diabetic animals of experiment B had four times more estradiol than did diabetics of experiment A when measured at 72 hr. None of the six diabetic animals killed at 72 hr had oviductal ova.

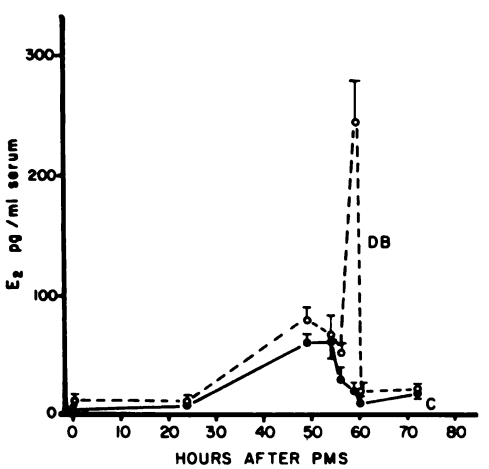

FIG. 2. The serum estradiol (E2) levels, determined by radioimmunoassay, for the control (C) and streptozotocin (DB)-treated rats of experiment A. All animals received 20 IU of PMS at time 0. Vertical lines indicate one SEM for groups of 10 rats.

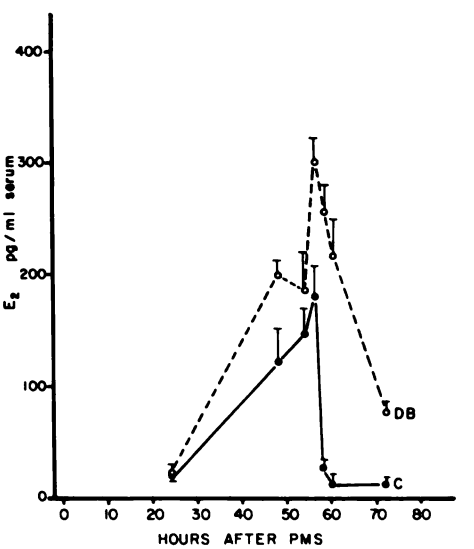

FIG. 3. Estradiol in the sera of females of experiment B. These animals were injected one month later than those of experiment A, with the same dose and lot of PMS. Nonfasting serum glucose levels were not different, nor was the pattern of change in E2 levels, from that of animals in experiment A, but the E2 levels were much higher in the females in this experiment.

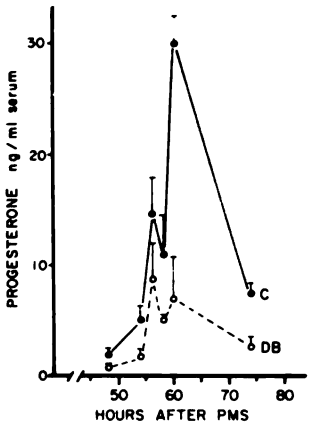

FIG. 4. Serum progesterone concentrations in the animals (10/group) of experiment A. While the pattern of change in the diabetic (DB) and control (C) animals was similar the latter had significantly more of this steroid when measured at 60 and 72 hr after injection of PMS.

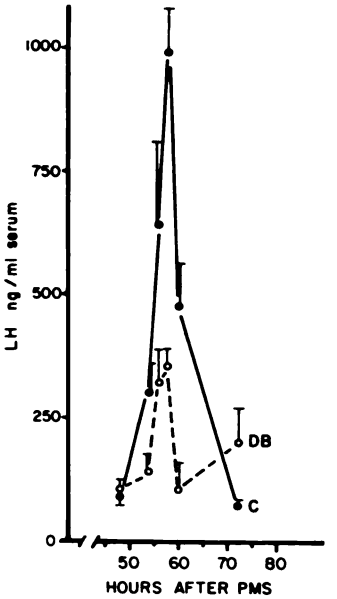

FIG. 5. The serum concentration of LH (as RP-1) in diabetic (DB) and control (C) rats of experiment A (E2 levels shown in Fig. 2).

Changes in serum progesterone concentrations were obtained for only the animals of experiment A (Fig. 4). The trend in the changes in concentration of this steroid in the two kinds of animals was the opposite of that seen for estradiol, i.e., the controls had the larger amount. Even after the decrease in serum progesterone which occurred between 60 and 72 hr in the controls the concentrations were more than double those of diabetic animals.

Changes in serum levels of LH for animals of experiments A and B are shown in Figs. 5 and 6, respectively. At 48 hr after PMS serum LH was the same in diabetic and control animals; in both the level was about four times that of immature controls not given PMS. The concentration rose quickly to a peak at 58 hr in the control and diabetic animals of experiment A but the quantitative differences were quite profound (Fig. 5). The level of LH in diabetic animals killed 72 hr after PMS was nearly three times that of controls ($P < 0.01$). The pattern of changes in serum LH in animals of experiment B were similar to those of experiment A but the peak level in B, which was only about half that found in animals of experiment A, was achieved 2 hr later. LH levels in diabetic animals of experiment B were not significantly different from those found in animals of experiment A.

Previous studies had demonstrated that steroid 17α-hydroxylase activity in the ovary decreased dramatically following an LH surge (11). Assay of the enzyme in immature diabetic females treated with PMS

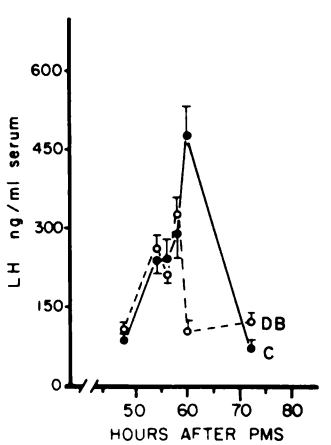

FIG. 6. Serum LH levels in females of experiment B, which had much higher levels of serum E2 (see Fig. 3).

revealed a pattern similar to that found in normal females. That is, after an initial decline in ovarian 17α-hydroxylase activity, an increase, which reached a peak at 60 hr was seen. The enzyme activity at 72 hr was significantly reduced, even though the ovaries of the two animals of the group which had ovulated were not included in the homogenate used for the assay.

In a third experiment, performed 3 months later and with a different lot of hormone, 7 of 8 control and 6 of 11 diabetic females had oviductal ova when examined at 72 hr after the injection of 20 IU of PMS (Table I). The number of ova shed was reduced by 66% ($P < 0.03$) in the animals treated with streptozotocin. Both kinds of females had received (sc) 0.1 ml of sesame seed oil 24 hr before autopsy. Injection of 1 mg of progesterone, dissolved in 0.1 ml of oil, 48 h after administration of PMS increased slightly (nonsignificantly) the number of ova shed by normal females. In the diabetic animals, however, ovulation was increased by the progesterone to the extent that 14 of the 16 ovulated. Furthermore, the number of ova released was increased by 135% ($P < 0.05$) compared to diabetic animals not receiving progesterone. With progesterone treatment the number of ova was not significantly different from the number released by normal females.

Discussion. The results of the present study confirm and extend the findings of Kirchick *et al.* (4, 5). These authors found that ovarian weight and estrogen production were similar in diabetic and normal immature rats when measured about 50 hr after a single injection of PMS. Further, they noted that serum progesterone levels were below normal in diabetic animals when measured either at 1200 or 2100 hr on the day of presumed proestrus. However, in contrast to their results with alloxan treatment, the present study has shown that animals made diabetic with streptozotocin produced an LH surge on the second day after injection of PMS and that some of the animals ovulated within 72 hr. The lower levels of serum LH in the diabetic animals may have accounted for the lowered percentage of animals ovulating and the reduced number of ova shed in some diabetic animals (Table I). In adult female rats with normal estrous cycles only 11 to 14% of the ovulatory LH surge on proestrus is required for ovulation (12). If this is also true for immature rats stimulated by PMS we would have expected a higher ovulatory rate because in both experiments with diabetic animals the peak LH levels were greater than 30% of those in control animals.

The decrease in ovarian 17α-hydroxylase activity seen in diabetic rats between 60 and 72 hr after PMS (Fig. 7) is another indication of an increase in LH release. This decrease in enzyme activity occurs in normal females between 48 and 60 hr after PMS, immediately following the LH surge. The enzyme activity continues to increase in hypophysectomized animals until at least

TABLE I. THE EFFECT OF PROGESTERONE UPON OVULATION IN NORMAL AND DIABETIC IMMATURE RATS INJECTED WITH PMS

Treatment	Group	No. of rats	Body weight	Serum glucose	Ova per animal	Ovulation rate (%)
Control	1	8	77.5 ± 2.1^a	86 ± 8^a	14.8 ± 4.4^a	87.5
Diabetic	2	11	62.9 ± 3.0^b	361 ± 21^b	4.9 ± 2.4^b	54.5
Control + progest.	3	14	80.4 ± 3.2^a	93 ± 2^a	21.5 ± 2.9^a	100
Diabetic + progest.	4	16	57.4 ± 2.3^b	349 ± 2^b	11.5 ± 3.4^a	87.5

Note. Results are means ± SEM. Diabetic animals were injected (iv) with streptozotocin (60 mg/kg) 3 days prior to injection (iv) of 20 IU of PMS. Progesterone (progest.) was injected (1 mg, sc) 48 hr after the PMS. Ova were flushed from the oviducts 72 hr after injection of PMS. Groups with the same superscript are not significantly different from each other, $P < 0.05$.

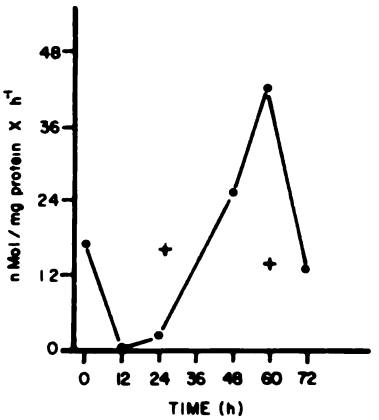

FIG. 7. Steroid 17α-hydroxylase activity in the microsomal fraction of ovaries from immature female rats made diabetic with streptozotocin; 17α[^{3}H]pregnenolone was the substrate. The pattern of change is similar to that for normal females (11) except that the decline associated with the LH surge is less pronounced and is delayed until 72 hr. Controls injected with saline (+) had no significant change in enzyme level. SEM for triplicate samples does not exceed the area covered by the symbols.

60 hr, and usually to 72 hr after injection of PMS before it gradually declines ((11) and unpublished data). In the present study, the ovarian hydroxylase activity of diabetic animals of experiment B at 48 hr post-PMS was 36.0 ± 0.4 nmole/mg protein/hr, while control ovaries had 32.0 ± 0.4. By 60 hr enzyme activity had decreased to 4.7 in controls but it had increased to 41.9 in the diabetic females. This delay in the ovarian response to LH may reflect the lower levels of this gonadotropin in the diabetic, and it may account for the high serum estrogen concentrations seen at 60 hr. However, it may also reflect an altered ovarian response to the gonadotropin because there is no difference in the timing of the LH surge between diabetic and normal females (Figs. 4, 5).

Kirchick *et al.* (5) concluded that the lack of an LH surge in alloxan diabetic rats was due to a pituitary insensitivity to hypothalamic gonadotropin-releasing hormone (GnRH). Furthermore, they demonstrated that the insensitivity was not secondary to a lack of estrogen stimulation of the pituitary. The present results also indicate that the ovaries of the diabetic respond to PMS by increasing production of estradiol. Possibly the estradiol levels were too high (Figs. 2 and 3) and rather than being stimulatory for LH release they may have become inhibitory, as shown by Wyss and Pincus (13).

The stimulatory effect of progesterone on ovulation in the diabetic rat suggests that the lack of this steroid may be a primary factor in the reduction of the LH surge. Grayburn and Brown-Grant (14) reported that a single injection of 0.5 mg of progesterone 50 hr after PMS increased the proportion of animals ovulating and the number of ova shed. The problem is understanding whether the low progesterone levels in the diabetic females are a consequence or a cause for the low LH levels. If an ovarian response was altered by the diabetic state and progesterone production was curtailed we would expect an inhibiting effect on ovulation at the ovarian level (15). On the other hand a reduction in LH release may account for the low progesterone levels which would result in the lack of an increasingly effective positive feedback loop. In either situation the administration of LH or progesterone would be expected to bring about normal ovulation rates; this is the case as shown by Kirchick *et al.* (4) and the results in Table I.

A role for the adrenal in loss of the ovulatory response in diabetics must be considered. Diabetes has long been recognized as a stress and to produce elevated serum levels of corticosteroids (16). Furthermore, in immature female rats, similar to those used in the present experiments, adrenal secretion of corticosterone was increased in response to PMS administration (17). The level was not high enough to inhibit ovulation, however, and after the LH surge in these animals adrenal function decreased dramatically: an ovarian product was responsible for the latter effect (17). If the progesterone from the heavily luteinized ovaries was responsible for lowering of the serum corticosterone levels, a point which has not been clearly established (see discussion in (17)), then the

stimulatory effect of progesterone on ovulation in the diabetic may involve yet another mechanism in addition to its effect on the release of LH and its direct effects upon the ovary. We have not measured serum corticosterone levels in the immature diabetics used in the present experiments.

In conclusion, the present study has shown that hyperglycemia induced by streptozotocin is not inconsistent with an LH surge or with ovulation. The difference in responses to those found with hyperglycemia induced by alloxan (4, 5) may be due to differences in the degree of pancreatic beta cell destruction induced by streptozotocin and consequently to the severity of the diabetes induced. Understanding the causes for ovulation reduction in uncontrolled diabetic animals will obviously require investigations into ovarian responses as well as those involving the hypothalamic–hypophyseal axis.

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1. Chiere RA, Pivetta OH, Foglia VG. Altered ovulation patterns in experimental diabetes. Fertil Steril 20:661–666, 1969.
2. Davis EM, Fugo NW, Lawrence KB. Effect of alloxan diabetes on reproduction in the rat. Proc Soc Exper Biol Med 66:638–641, 1947.
3. Lawrence DM, Contopoulos AN. Reproductive performance in the alloxan diabetic female rat. Acta Endocrinol 33:175–184, 1960.
4. Kirchick HJ, Keyes PL, Frye BE. Etiology of anovulation in the immature diabetic rat treated with pregnant mare's serum gonadotropin: Absence of the preovulatory LH surge. Endocrinology 102:1867–1873, 1978.
5. Kirchick HJ, Keyes PL, Frye BE. An explanation for anovulation in immature alloxan-diabetic rats treated with pregnant mare's serum gonadotropin: Reduced pituitary response to gonadotropin-releasing hormone. Endocrinology 105:1343–1349, 1979.
6. Hoftiezer V, Carpenter A.-M. Comparison of streptozotocin and alloxan induced diabetes in the rat, including volumetric quantitation of the pancreatic islets. Diabetologia 9:178–184, 1973.
7. Mallampati RS, Johnson DC. Serum and pituitary prolactin, LH and FSH in androgenized female and normal male rats treated with various doses of estradiol benzoate. Neuroendocrinology 11:46–56, 1973.
8. Johnson DC. Maintenance of functional corpora lutea in androgenized female rats treated with PMSG. J Reprod Fertil 56:263–269, 1979.
9. Exley D, Johnson AW, Dean PDG. Antisera highly specific for 17β-estradiol. Steroids 18:605–620, 1971.
10. Grotjan HE Jr, Johnson DC. Temporal variations in reproductive hormones in the immature male rat. Proc Soc Exp Biol Med 152:381–384, 1976.
11. Johnson DC. Temporal changes in ovarian steroid 17α-hydroxylase in immature rats treated with pregnant mare's serum gonadotropin. Proc Soc Exper Biol Med 159:484–487, 1978.
12. Grieg F, Weisz J. Preovulatory levels of luteinizing hormone, the critical period and ovulation in rats. J Endocrinol 57:235–245, 1973.
13. Wyss HT, Pincus G. Effect of pregnant mare's serum gonadotropin, estradiol and progesterone on superovulation in the immature rat. Endocrinology 75:586–591, 1964.
14. Grayburn JA, Brown-Grant K. The role of oestrogen in the induction of ovulation in the gonadotrophin-treated immature rat. J Endocrinol 42:409–416, 1968.
15. Takahashi M, Ford JJ, Yoshinaga K, Greep RO. Induction of ovulation in hypophysectomized rats by progesterone. Endocrinology 95:1322–1326, 1974.
16. Devecerski MC, Frawley TF. Adrenal steroid production in rats with alloxan diabetes. Endocrinology 73:368–391, 1963.
17. Ramaley JA, Olson J. Adrenal function in rats given PMS before puberty: Response to ether stress. Neuroendocrinology 14:1–13, 1974.

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Carbohydrate Metabolism during the Postprandial Intestinal Hyperemia (41501)

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Abstract. Intestinal carbohydrate uptake and utilization were studied before and during the perfusion of the canine jejunal lumen with food. Under conditions of normal oxygen consumption (1.8 ml O_2/min/100 g) and blood flow (46.4 ± 3.5 ml/min/100 g), intestinal glucose uptake was low (2 mg/min/100 g) and glucose utilization was aerobic. When food was present in the lumen, there was a 15% increase in intestinal metabolism, as measured by oxygen consumption, while lactic acid production increased threefold. This increase in lactic acid production was not due to a shift in glycolysis toward lactic acid synthesis as the venous lactic acid/pyruvic acid concentration ratio actually decreased. Intestinal glucose utilization was offset by carbohydrate absorption as glucose uptake from arterial blood decreased. The data suggest that a relatively greater proportion of intestinal energy demand is met by glycolysis during the absorption of nutrients than at rest and supports the findings of other investigators that a portion of absorbed glucose is metabolized. In addition, the study indicates that intestinal glucose metabolism does not terminate in lactic acid synthesis as suggested by a previous study.

Recent studies indicate that the postprandial intestinal hyperemia is limited to those portions of the small intestine which have been exposed to chyme (1, 2). It has also been shown that the constituents of chyme which are responsible for this hyperemia are the products of enzymatic food digestion (3). Of these, glucose has been shown to increase jejunal blood flow and oxygen consumption when placed in the lumen in solution (4, 5). A number of studies have indicated that a portion of the glucose which is absorbed by the small intestine is metabolized (6–9) and Sit *et al.* (4) have shown that this metabolism is responsible for a significant portion of the glucose-induced increase in jejunal blood flow and oxygen consumption.

In two studies of glucose absorption (8, 9), a small fraction of the absorbed glucose appeared as lactic acid in the venous blood; however, it is not known if this lactic acid represents the end product of intestinal glucose metabolism, as suggested by Lester and Grim (10), or simply the by-product of aerobic glycolysis. In addition, in previous studies of intestinal glucose absorption, glucose was the only nutrient present in the lumen and it is not certain to what extent absorbed glucose is utilized for intestinal energy production when other substrates are available. Therefore, in this study we examined the nature of carbohydrate utilization in the canine jejunum when either saline or a nutrient solution containing fat, carbohydrate, and protein were present in the lumen.

Materials and Methods. Adult mongrel dogs of either sex (15–25 kg; N = 12) were fasted for 24 hr, anesthetized with sodium pentobarbital (30 mg/kg, iv) and ventilated with a positive pressure Harvard respirator to ensure normal arterial pH (7.38–7.43). Following a midline abdominal incision, a loop of the jejunum about 30 cm distal to the ligament of Treitz was exteriorized and a segment drained by a single vein was selected. After administration of sodium heparin (6 mg/kg), the vein draining the segment was cannulated and the venous effluent was directed through an extracorporeal electromagnetic flow transducer

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(Biotronex BLC-2048-E04 connected to a Biotronex BL-610 flowmeter) to a venous reservoir containing 200 ml of a dextran solution (6% in normal saline). The volume of the reservoir was maintained at 200 ml by returning the contents to the femoral vein at a rate equal to the venous outflow.

A piece of rubber tubing was placed in each end of the jejunal segment and tied in place. Both ends of the segment were tied and cut and the mesentery was cut to exclude collateral flow. The segment was covered with a plastic sheet and kept at 37° with a heat lamp. The tubing in the proximal end of the segment was connected via a Masterflex pump (Cole Parmer, Chicago, Ill.) to a reservoir containing normal saline at 37°. Warm saline was perfused through the segment at a rate of 6 ml/min. The tubing in the distal end of the segment served as a drain for the segment.

The arterial–venous oxygen ($A-VO_2$) content difference across the segment was measured continuously by perfusing femoral arterial blood and a portion of the venous outflow from the segment through the cuvettes of an $A-VO_2$ content difference analyzer (AVOX Systems, San Antonio, Tex.). The analyzer was previously calibrated with a Lex-O_2 Con TL oxygen content analyzer (Lexington Instruments, Waltham, Mass.) and the output signal was recorded continuously on a Hewlett-Packard recorder (Waltham, Mass.). The signal from the flowmeter was also recorded continuously and the flow transducer was calibrated periodically during the course of the experiment by measuring the venous outflow with a graduated cylinder and stopwatch. Systemic arterial pressure was monitored continuously through a femoral artery cannula.

After surgery had been completed, saline was perfused through the lumen until blood flow and the $A-VO_2$ content difference had reached a steady state (approx. 30 min). At that time, duplicate blood samples were taken from the femoral artery and the venous outflow of the segment for the measurement of blood pH, pO_2, pCO_2, lactic acid, pyruvic acid, and glucose. The perfusate was then changed to a mixture of digested food and bile, at 37°, and, when a new steady state had been achieved, duplicate arterial and venous blood samples were taken as before. The animal was then killed with an overdose of anesthetic and the segment was excised, trimmed of all mesentery, and weighed. Blood flow, oxygen consumption, glucose uptake, and lactic acid and pyruvic acid production were expressed in units per 100 grams tissue weight.

Care was taken during the collection of the arterial and venous blood samples for the measurement of pH, pO_2, and pCO_2 to ensure that the samples were not contaminated with room air. Arterial and venous blood samples for the determination of arterial and venous glucose, lactic acid, and pyruvic acid concentrations were collected on ice in test tubes containing 10 mg of NaF. The NaF served to inhibit red blood cell glycolysis. The portion of blood to be used for the measurement of the pyruvic acid concentration was pipetted from each blood sample immediately after collection and vortexed with an 8% perchloric acid solution to precipitate the blood proteins. This served to block enzymatic degradation of the pyruvic acid.

The arterial and venous blood gases were measured with a BMS 3 MK II Blood MicroSystem and Acid–Base Analyzer (London Co., Cleveland, Ohio). The glucose concentration in each blood sample was measured in a YSI Glucose Analyzer (YSI, Yellow Springs, Ohio). The pyruvic acid and lactic acid concentrations were measured spectrophotometrically using a lactic acid dehydrogenase assay (Sigma Chemical Co., St. Louis, Mo.).

The oxygen consumption of the segment was determined by multiplying the appropriate values from the blood flow and $A-VO_2$ content difference recordings after correcting for the lag time between the recordings due to the distance between monitoring points. Glucose uptake was calculated as the product of the arterial–venous concentration difference and jejunal blood flow while lactic acid and pyruvic acid production were taken as the product of the venous–arterial concentration difference and blood flow.

The nutrient solution used in this study

TABLE 1. MEAN SYSTEMIC ARTERIAL PRESSURE (SAP) AND JEJUNAL BLOOD FLOW (BF), $A-VO_2$ CONTENT DIFFERENCE (ΔAVO_2), OXYGEN CONSUMPTION ($\dot{V}O_2$), AND VENOUS HEMATOCRIT (Hct) BEFORE AND DURING THE PERFUSION OF FOOD THROUGH THE LUMEN

	Lumen contents		
	Saline	Food	Food−saline
SAP (mm Hg)	117 ± 5	117 ± 5	0 ± 3
BF (ml/min/100 g)	46.4 ± 3.5	54.6 ± 4.2	8.2 ± 1.3*
ΔAVO_2 (ml O_2/100 ml)	4.1 ± 0.3	4.0 ± 0.3	−0.1 ± 0.1
$\dot{V}O_2$ (ml O_2/min/100 g)	1.78 ± 0.08	2.06 ± 0.10	0.28 ± 0.05*
Hct	41 ± 1	40 ± 2	−1 ± 2

Note. Values are means ± SEM; $N = 12$.
* $P < 0.05$.

contained equal parts by weight of fat, protein, and carbohydrate. Fifty grams of this food mixture was combined with 500 mg of a pancreatic enzyme preparation (Viokase, Viobin Co., Monticello, Ill.) and mixed at room temperature for 5 hr in 400 ml of 0.1 *N* $NaHCO_3$. Prior to each experiment, nine parts of the digested food solution were mixed with one part of bile from the dog's gallbladder. The osmolarity of the final solution was adjusted to 300 ± 20 mosmole/kg with NaCl or distilled water and the pH was adjusted to 7.0 ± 0.2 with HCl or NaOH.

The data are expressed as the mean ± SEM and comparisons between treatments were made using Student's *t* test modified for paired comparisons. Statistical significance was set at *P* values less than 0.05.

Results. As seen in Table I, mean systemic arterial pressure was 117 ± 5 mm Hg and it did not change during the course of the experiment. Also, as shown in Table I, jejunal blood flow was 46.4 ± 3.5 ml/min/100 g and the $A-VO_2$ content difference was 4.1 ± 0.3 ml O_2/100 ml when saline was in the lumen yielding an oxygen consumption of 1.78 ± 0.08 ml O_2/min/100 g. Glucose uptake was 1.96 ± 0.24 mg/min/100 g and jejunal lactic acid and pyruvic acid production were 0.26 ± 0.10 mg/min/100 g and 0.059 ± 0.012 mg/min/100 g, respectively (Fig. 1). The venous lactic acid/pyruvic acid concentration ratio (*L/P* ratio) was 26 ± 2 (Fig. 1). This indicates that when saline is present in the lumen, jejunal glucose metabolism is aerobic and does not terminate in lactic acid synthesis (11).

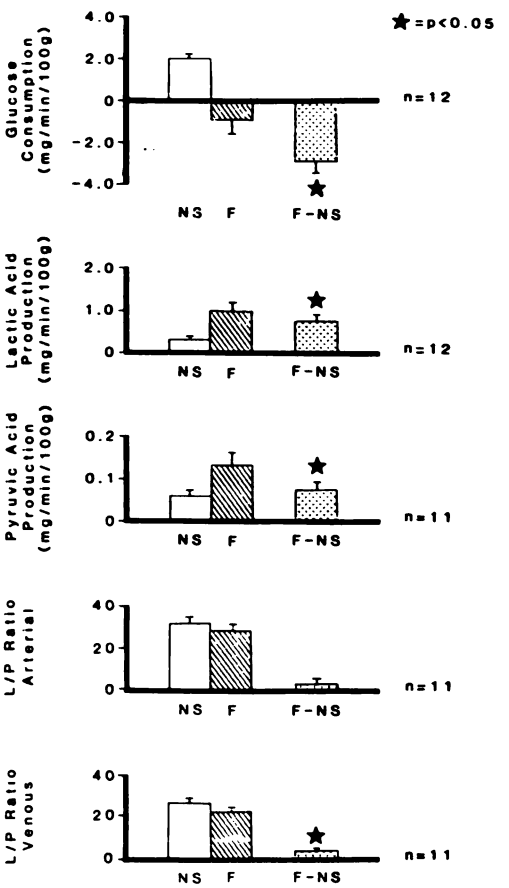

FIG. 1. Mean ± SEM of jejunal glucose consumption and lactic acid and pyruvic acid production and the arterial and jejunal venous lactic acid/pyruvic acid concentration ratios with either normal saline (NS) or food (F) in the lumen.

TABLE II. ARTERIAL AND VENOUS BLOOD pH, pO_2, AND pCO_2 BEFORE AND DURING PERFUSION OF THE JEJUNAL LUMEN WITH FOOD

	Arterial	Venous	A-V Difference
	Saline in the lumen		
pH	7.41 ± 0.01	7.40 ± 0.01	0.01 ± 0.004*
pO_2	86 ± 4	37 ± 1	49 ± 4*
pCO_2	33 ± 1	34 ± 1	−1 ± 1
	Food in the lumen		
pH	7.41 ± 0.01	7.38 ± 0.01	0.03 ± 0.04*,†
pO_2	85 ± 4	38 ± 2	47 ± 3*
pCO_2	34 ± 2	38 ± 2	−4 ± 1*,†

Note. Values are means ± SEM; $N = 12$.
* $P < 0.05$.
† $P < 0.05$ relative to the corresponding value with saline in the lumen.

The arterial pH was 7.41 ± 0.01, the pO_2 was 86 ± 4 mm Hg and the pCO_2 was 33 ± 1 mm Hg (Table II). The venous pH (7.40 ± 0.01) was only slightly less than arterial pH but the difference was statistically significant. In addition, the venous pO_2 (37 ± 1 mm Hg) was significantly less than arterial pO_2 but there was no significant arterial−venous pCO_2 difference. The venous hematocrit was 41 ± 1 (Table I).

When food was perfused through the lumen, there was a significant increase in jejunal blood flow and oxygen consumption (Table I). Jejunal blood flow increased 8.2 ± 1.3 ml/min/100 g (18 ± 3%) to 54.6 ± 4.2 ml/min/100 g. Oxygen consumption increased 0.28 ± 0.05 ml O_2/min/100 g to 2.06 ± 0.10 ml O_2/min/100 g, an increase of 15 ± 3%. The increased demand for oxygen was met entirely by the increase in blood flow as the $A-VO_2$ content difference did not change (Table I).

The luminal presence of food significantly decreased jejunal glucose uptake from the blood to −0.88 ± 0.63 mg/min/100 g, a decrease of 2.84 ± 0.68 mg/min/100 g (Fig. 1). At the same time both lactic acid and pyruvic acid production increased significantly. Lactic acid production increased to 1.02 ± 0.18 mg/min/100 g, an increase of 0.76 ± 0.17 mg/min/100 g or nearly 300%. Pyruvic acid production increased by 0.07 ± 0.024 mg/min/100 g to 0.128 ± 0.027 mg/min/100 g. There was no significant change in the arterial L/P ratio while the venous L/P ratio decreased slightly (−4 ± 1).

There were no significant changes in arterial pH, pO_2, or pCO_2 when food was present in the lumen (Table II); however, there were significant increases in the arterial−venous pH and pCO_2 differences. The arterial−venous pCO_2 difference increased from −1 ± 1 to −4 ± 1 mm Hg and the arterial−venous pH difference increased from 0.01 ± 0.004 to 0.03 ± 0.004, reflecting the increased CO_2 and lactic acid production. There was no significant change in either the arterial−venous pO_2 difference (Table II) or the venous hematocrit (Table I).

Discussion. Recent studies indicate that when glucose is placed in the lumen of the small intestine, 15−40% of the glucose which is absorbed does not appear in the venous blood (6, 7, 9). This net loss of glucose across the intestinal wall has been attributed to the utilization of absorbed glucose for energy production within the mucosal layer (8−10). This concept is supported by the fact that there is an increase in the venous lactic acid concentration during glucose absorption which is equivalent to 5−10% of the absorbed glucose (8, 9). It has even been suggested that this intracellular conversion of glucose to lactic acid in the small intestine and its subsequent release into the portal circulation where it can be utilized as a substrate for hepatic gluconeogenesis may serve as another mechanism of intestinal glucose absorption (12).

In this study, we examined the nature of jejunal glucose metabolism with either saline or food in the lumen in order to determine if glucose utilization increases when a variety of nutrients are present in the lumen. Furthermore, we wished to determine if lactic acid is the end product of intestinal glucose metabolism as suggested by Lester and Grim (10).

The carbohydrate used in this study was sucrose (4 g/100 ml) which consists of one molecule each of glucose and fructose and is broken down to its constituents by the intestinal brush border enzyme, sucrase.

Although fructose was not measured in this study, 60–95% of absorbed fructose is converted to either glucose or lactic acid within the small intestine (9, 13–15) so that, in this experiment, venous glucose and lactic acid represent at least 80% of the absorbed carbohydrate.

The data indicate that, when saline is present in the lumen, there is a low level of glucose uptake and lactic acid and pyruvic acid production. These findings support those of Shoemaker *et al.* (9), who reported a control lactic acid production of only 2.8 mg/min for the entire nonhepatic splanchnic area in conscious dogs, and contradict those of Lester and Grim (10), who found that in canine jejunal mucosa 80% of metabolized glucose was converted to lactic acid *in vitro*. The reasons for this discrepancy in results between *in vivo* and *in vitro* studies are not readily apparent. It is possible that portions of the tissues in the *in vitro* studies were anoxic and relied on anaerobic glycolysis for energy production; however, Wilson (12) presents strong evidence against such a possibility in similar studies using everted rat gut sacs.

It is interesting to note that Wilson has also reported a sixfold increase in lactic acid production by everted rat ileal sacs when glucose was placed on the mucosal rather than the serosal side (12). Therefore, it is possible that the difference between the *in vivo* and *in vitro* studies of canine jejunal glucose metabolism may be due to the fact that in the *in vitro* studies glucose was exposed to the mucosal surface while in the control period of the *in vivo* studies it was not. It is not clear, however, if these results are applicable to other species as the nature of glucose metabolism in the rat and mouse intestines is considerably different from other species such as the guinea pig, hamster, and rabbit (12).

When food was perfused through the lumen, there was an increase in jejunal oxygen consumption, a decrease in glucose uptake from arterial blood, and an increase in both lactic acid and pyruvic acid production. As it was not possible to accurately measure the disappearance of carbohydrate from the lumen in these studies, the conclusions which may be drawn from the available data must be based on certain assumptions and are therefore tentative.

In the saline-perfused jejunal loop, glucose was taken up from the blood at a rate of 66 μmole $C \cdot min^{-1} \cdot 100\ g^{-1}$. Of this, lactic acid and pyruvic acid production accounted for 11 μmole $O_2 \cdot min^{-1} \cdot 100\ g^{-1}$. Oxygen consumption during this period was 80 μmole $O_2 \cdot min^{-1} \cdot 100\ g^{-1}$. If the remaining 55 μmole $C \cdot^{-1} \cdot 100\ g^{-1}$ were converted entirely to CO_2, then 70% of the oxygen consumed by the segment was used for glucose metabolism. However, Lester and Grim (10) have reported that only 50% of jejunal oxygen consumption is used for carbohydrate metabolism *in vitro*. If these results are representative of conditions *in vivo*, then approximately 25% of the glucose metabolized by the saline-perfused jejunal loop was converted to products other than CO_2, lactic acid, and pyruvic acid.

When food was perfused through the lumen of the jejunal loop, there was a threefold increase in the rate of lactic acid production by the segment. This increase could have been due to either a shift in carbohydrate metabolism toward lactic acid synthesis while total carbohydrate metabolism remained unchanged or to an increase in the overall rate of carbohydrate metabolism. The former conclusion would support Wilson's findings that the metabolic fate of glucose depends upon which surface of the cell it must cross, i.e., serosal or mucosal. However, a shift in carbohydrate metabolism toward lactic acid synthesis is usually associated with an increase in the venous *L/P* ratio (11). This did not occur, rather the venous *L/P* ratio decreased slightly in this study. Therefore, it would seem likely that the increased rate of lactic acid synthesis represents an increase in the overall rate of carbohydrate metabolism.

There is some evidence to support the conclusion that a portion of absorbed glucose is metabolized by the canine jejunum in a recent study by Sit *et al.* (4). In that study, the authors examined the effect of placing either glucose or 3-*O*-methyl glucose in the jejunal lumen on jejunal oxygen

consumption. While both glucose and 3-*O*-methyl glucose are actively transported by the mucosal epithelia, only glucose is metabolized by intestinal tissues. Sit *et al.* found that although the jejunal loops absorbed equal amounts of each carbohydrate, only glucose increased intestinal oxygen consumption. The authors attributed this difference in response to metabolism of absorbed glucose.

If jejunal carbohydrate metabolism did increase when food was perfused through the lumen, the amount of absorbed carbohydrate which was metabolized depends upon which assumptions regarding the nature of intestinal carbohydrate metabolism which we have discussed previously are correct. If, as Lester and Grim have reported (10), 50% of intestinal oxygen consumption is directed toward carbohydrate metabolism, then total carbohydrate utilization in the food-perfused loops was 84 μmole $C \cdot min^{-1} \cdot 100\ g^{-1}$ (38 μmole $C \cdot min^{-1} \cdot 100\ g^{-1}$ converted to lactate/pyruvate; 46 μmole $C \cdot min^{-1} \cdot 100\ g^{-1}$ converted to CO_2). This would represent an increase in the rate of carbohydrate metabolism over control of 18 μmole $C \cdot min^{-1} \cdot 100\ g^{-1}$ or 16% of the absorbed glucose. The rate of glucose absorption in this case being equal to the sum of the decrease in the rate of glucose uptake from the blood (96 μmole $C \cdot min^{-1} \cdot 100\ g^{-1}$) and the assumed increase in the rate of carbohydrate metabolism (18 μmole $C \cdot min^{-1} \cdot 100\ g^{-1}$). If 70% of the oxygen consumed were directed toward carbohydrate metabolism or if glucose were degraded to compounds other than lactic acid or pyruvic acid, then the percentage metabolized would be even greater.

Although resting glucose utilization by the small intestine is low (4, 9, 10), it should not seem surprising that the rate of glycolysis should increase during periods of nutrient absorption. There is no reason to believe that absorbed nutrients are compartmentalized as they pass through the cells of the mucosa and therefore one would expect that a portion of those nutrients would be metabolized according to the laws of mass action. If the increase in the rate of lactic acid production is proportional to the overall increase in the rate of glycolysis, then it would appear that the relative proportion of energy demand met by glycolysis increased when food was present in the lumen as there was a threefold increase in the rate of lactic acid production while total intestinal metabolism increased only 15%. Again, this should not seem surprising as glucose is available for immediate utilization while the fats, tripeptides, dipeptides, and amino acids require additional catabolism before they can be utilized for energy production.

The results of this study indicate that when blood flow and oxygen delivery are adequate, there is a low level of intestinal glucose metabolism which does not appear to terminate in lactic acid synthesis. When food is present in the lumen, there is an apparent increase in the rate of glucose utilization which is proportionally greater than the overall increase in the rate of intestinal metabolism. The demand for glucose by the small intestine is apparently offset by carbohydrate absorption as glucose uptake from the arterial blood actually decreases. These findings lend support to the conclusions of other investigators (4, 6–9) that a portion of the glucose absorbed from the lumen of the small intestine is metabolized and suggests that metabolism is directed through the Kreb's cycle rather than terminating in lactic acid synthesis.

1. Gallavan RH Jr, Chou CC, Kvietys PR, Sit SP. Regional blood flow during digestion in the conscious dog. Amer J Physiol 238:H220–H225, 1980.
2. Chou CC, Hsieh CP, Yu YM, Kvietys P, Yu LC, Pittman R, Dabney JM. Localization of mesenteric hyperemia during digestion in dogs. Amer J Physiol 230:583–589, 1976.
3. Chou CC, Kvietys P, Post J, Sit SP. Constituents of chyme responsible for postprandial intestinal hyperemia. Amer J Physiol 235:H677–H682, 1978.
4. Sit SP, Nyhof R, Gallavan R, Chou CC. Mechanisms of glucose-induced hyperemia in the jejunum. Proc Soc Exp Biol Med 163:273–277, 1980.
5. Shepherd AP. Intestinal capillary blood flow during metabolic hyperemia. Amer J Physiol 237:E548–E554, 1979.

6. Parsons DS. Prichard JS. A preparation of perfused small intestine for the study of absorption in amphibia. J Physiol 198:405–434. 1968.
7. Balent GA. Kiss ZF. Varkonyi T. Wittmann T. Varro. V. Effects of prostaglandin E_2 and $F_{2\alpha}$ on the absorption and portal transport of sugar and on the local intestinal circulation. Prostaglandins 18:265–268. 1979.
8. Wilson TH. Wiseman G. The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J Physiol 123:116–128. 1954.
9. Shoemaker WC. Yanof HM. Tusk LN. Wilson TN. Glucose and fructose absorption in the unanesthetized dog. Gastroenterology 44:654–663. 1962.
10. Lester RG. Grim E. Substrate utilization and oxygen consumption by canine jejunal mucosa in vitro. Amer J Physiol 229:139–143. 1975.
11. Williamson JR. Glycolytic control mechanisms. J Biol Chem 244:5026–5036. 1966.
12. Wilson TH. The role of lactic acid production in glucose absorption from the intestine. J Biol Chem 222:751–763. 1956.
13. Bollman JL. Mann FC. The utilization of fructose following complete removal of the liver. Amer J Physiol 96:683–695. 1931.
14. Öckerman PA. Lunborg H. Conversion of fructose to glucose by human jejunum absence of galactose-to-glucose conversion. Biochim Biophys Acta 105:34–42. 1956.
15. Kiyasu JY. Chaikoff IL. On the manner of transport of absorbed fructose. J Biol Chem 224: 935–946. 1957.

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Golgi Complex Function in the Excretion of Renal Kallikrein (41502)

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Abstract. A Golgi complex rich-fraction containing both *N*-acetylglucosamine galactosyltransferase and kallikrein activity has been isolated from kidney of rats previously treated with colchicine, a secretion inhibitor, followed by the administration of high-sodium solutions, to stimulate biosynthesis or activation of renal kallikrein. After the treatment, *N*-acetylglucosamine galactosyltransferase and kallikrein activities were increased in the Golgi complex, about 18- and 24-fold, respectively, as compared to the homogenate. Low kallikrein activity was found in the crude light mitochondrial fraction from treated animals, whereas a high level of activity was observed in the microsomal fraction. The inverse situation was found in rats treated only with high-sodium solution. Results suggest that kallikrein is probably transported by microsomal elements, particularly by the Golgi complex. Furthermore, the evidence seems to indicate that the kallikrein activity reported in the plasma membrane and/or in the lysosomal fraction is due to kallikrein secretion, in the form of intact granules, which have sedimented with these two fractions.

Glandular kallikreins are a group of related kinin-forming enzymes present at least in the major exocrine glands and in the kidney. Previous studies on the role of the kallikrein–kinin system in the kidney identify kinins as potent vasodilators which cause natriuresis and diuresis when injected into the renal artery (1).

It is known that urinary kallikrein is a glycoproteic enzyme and that the renal kallikrein is secreted into the urine at the level of the distal tubule (2, 3). Moreover, both the renal and urinary enzymes are immunologically and electrophoretically similar (4). Renal kallikrein is also a glycoprotein and it is found in cells in the form of dense granules, which are transported to the extracellular space by some undefined mechanism by microsomal elements (5).

The localization of renal kallikrein is subject of dispute. Some authors refer it to the lysosomal fraction (6, 7), others to the microsomal fraction (8, 9), and still others to the plasma membrane fraction of kidney homogenates (10). In an attempt to clarify the apparently conflicting findings, we have isolated and characterized different subcellular fractions from rat kidney, using both differential and density gradient centrifugation techniques. Our approach was to increase the intracellular amount of kallikrein by a combination of two treatments: salt loading, that we showed increases the activation or the biosynthesis of this enzyme (11), and colchicine treatment, that inhibits the liver secretion by a reduction of the microtubule content of the cells (12, 13).

Our results indicate that Golgi complex participates in the intracellular transport and probably in the biosynthesis and/or activation of renal kallikrein.

Material and Methods. Female Sprague–Dawley rats, 200 to 300 g, fed *ad libitum* were used. Animals were treated with colchicine and then loaded with a 0.342 *M* saline solution at 5% body weight by gavage (14). The unanaesthetized animals were decapitated 2, 4, 8, and 10 min after treatment, then exsanguinated, and their kidneys quickly removed, decapsulated,

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and collected in ice-cold 0.25 *M* sucrose. Colchicine was given ip by way of two consecutive injections, 60 and 15 min before killing the animals. Two doses of colchicine were used in two groups of rats: 0.5 and 1.0 mg per 100 g body weight, respectively. UDP-Gal (Calbiochem, Los Angeles, Calif.) and UDP-Gal uniformly labeled with ^{14}C in the sugar moiety (New England Nuclear Corp., Boston, Mass.) were used. The latter was diluted with carrier to a specific activity of about 1 mCi/nmole.

Cell fractions were prepared from a pool of kidneys blotted, weighed, and minced with scissors. Centrifugations were carried out in Beckmann ultracentrifuge, and all operations were carried out at 0–4°. The Golgi complex fraction was prepared by the method previously described (15), with some modifications. About 10 g of minced tissue was suspended in 2 vol of 52% sucrose containing 0.1 *M* sodium phosphate, pH 7.1, and homogenized with three full strokes at 1000 rpm using a 50-ml glass Potter–Elvehjem type homogenizer with an i.d. of exactly 1 in and a Teflon pestle machined to a diameter of 0.974 in. The homogenization was repeated using a pestle with a diameter of 0.982 in. The homogenate was filtered through four layers of cheesecloth and adjusted to 43.7% sucrose with homogenizing medium. Seven to nine milliliters of homogenate were placed in a tube and overlaid with sufficient 37.8% sucrose to bring the total volume to 15.4 ml. This was then successively overlaid with 6.3 ml of 36% sucrose then with 6.3 ml of 33% sucrose and finally with 7 ml 29% sucrose. The step gradient was then centrifuged for 60 min at 25,000 rpm in a SW 25.1 rotor. The Golgi complex rich-fractions 1 and 2 were obtained from the material appearing at the 29/33% and 33/36% sucrose interfaces, respectively. The fractions were diluted with 1/2 vol of cold distilled water and the membranes recovered by centrifugation at 30,000 rpm for 1 hr in a 30 rotor.

The light crude mitochondrial, the light mitochondrial, and the microsome fractions were prepared by the methods of Stein *et al.* (16) as modified by Fleischer and Kervina (17). About 10 g of minced tissue was suspended in 4 vol of 0.25 *M* sucrose solution containing 0.01 *M* Hepes, pH 7.4, and homogenized with three full strokes at 1000 rpm using a 50-ml Potter–Elvehjem type homogenizer (i.d. of glass vessel, 1 in) and a Teflon pestle machine to a diameter of 0.974 in and followed with three full strokes with a pestle machined to 0.988 in. The homogenate was filtered through a 110-mesh nylon monofilament bolting cloth. The homogenate was differentially centrifuged, and a low spin pellet (1000*g* for 10 min), a light crude mitochondrial, a microsomal, and a soluble fraction were successively isolated. The light mitochondrial fraction was obtained by further purification of the crude fraction resuspended in 0.25 *M* sucrose–0.01 *M* Hepes–0.001 *M* EDTA, pH 7.4, and recentrifuged for 10 min at 18,000 rpm in a 40 rotor. The upper light portion (mostly heavy microsomes) of the pellet was separated and discarded. The lower brown portion, enriched in mitochondria, was resuspended in the same solution and centrifuged again for 10 min at 14,000 rpm with the same rotor. The residual upper layer was again removed. A lysosomal fraction was obtained from the light crude mitochondrial fraction by step sucrose gradient centrifugation according to the method of Maunsbach (18). The fraction, resuspended in 0.3 *M* sucrose–0.001 *M* EDTA, pH 7.0, was layered on a linear sucrose gradient (0.3–2.1 *M*) containing 0.001 *M* EDTA and was centrifuged for 180 min at 24,500 rpm in a SW 25.1 rotor. The most dense band is enriched in lysosomes.

Microsomes were further fractionated into a smooth and a rough fraction by a modification (17) of the method of Dallner (19) as described for liver. Microsomes, resuspended with 0.25 *M* sucrose–0.015 *M* CsCl solution, were layered into tubes containing 1.3 *M* sucrose–0.015 *M* CsCl and centrifuged for 180 min at 49,000 rpm in a 50 rotor. The rough microsomes sediment as a pellet and the smooth microsomes remain at the interface.

Glucose-6-phosphatase was determined according to the method of Swanson (20), except that incubations were carried out for

5 and 10 min. Succinate–cytochrome *c* reductase activity was determined as previously described (21). Acid phosphatase activity, using β-glycerophosphate as substrate, was measured by the method of Besseys *et al.* (22), except that inorganic phosphate was measured using the method of Chen *et al.* (23). Galactosyltransferase was determined as previously described by Fleischer (24).

Kallikrein activity was measured using two different methods:

(a) By its stimulating effect on uterine contractility (25), using bradykinin as a standard. Activity is expressed as kallikrein equivalent to nanograms of bradykinin per milligram of protein. Trasylol (Aprotinin), was used as inhibitor of the stimulating effect of the enzyme in the bioassay (25).

(b) By the esterase activity shown by kallikrein, using benzoyl-L-arginine ethyl ester (BAEE) as substrate (6). The colorimetric reaction was measured by the method of Brown (26) and expressed as micromoles BAEE hydrolyzed per minute per milligram of protein.

Proteins were determined by Lowry's procedure (27), using crystalline bovine serum albumin as a standard. Phosphorus was determined by the method of Chen *et al.* (23).

Undiluted aliquots of the sucrose interphase containing the Golgi complex were fixed with 1/10th of 25% glutaraldehyde made up in 0.2 *M* sodium cacodylate at pH 7.4, immediately after isolation from the step gradient. The other cell fractions were fixed by treating an aliquot with an equal volume of 5% glutaraldehyde in 0.25 *M* sucrose and 0.2 *M* sodium cacodylate, pH 7.4. After standing overnight in the refrigerator, the samples were centrifuged at 20,000 rpm for 15 min in a 40 rotor and the supernatant discarded. Pellets were then washed twice by suspension in 0.25 *M* sucrose and recentrifuged. Finally the pellets were fixed with 1% osmium tetroxide, dehydrated, embedded, and sectioned as previously described (28).

Results. The activity of kallikrein in kidney homogenate, in light crude mitochondrial, and in microsomal fractions isolated by differential centrifugation, is shown in Table I. The kidney homogenate of loaded rats shows the highest kallikrein activity 8 min after salt loading. This activity is five times higher than that obtained in nonloaded rats. Ten minutes after salt loading, the kallikrein activity shows only a twofold increase with respect to that of nonloaded rats. The activity measured 4 min after salt loading in light crude mitochondrial and in microsomal fractions, isolated from the same homogenate, increased 17- and 26-fold, respectively, as compared to the values obtained in the same fractions from nonloaded rats. After 8 min of salt loading the corresponding values were increased 56- and 42-fold, respectively. It is necessary to indicate that kallikrein activity in homogenate as well as in light crude mitochondrial and in microsomal fractions, from kidneys of rats sacrificed 2, 4, 8, and 10 min after sham gavage submission, were similar to that of nonloaded rats.

Table II describes the effect of a pre-

TABLE I. RENAL KALLIKREIN ACTIVITY OF LIGHT CRUDE MITOCHONDRIAL AND MICROSOMAL FRACTIONS OBTAINED FROM SODIUM-LOADED RATS

		Fractions	
	Homogenate	Mitochondrial	Microsomal
Nonloaded rat	1.07 ± 0.10	0.75 ± 0.08	0.23 ± 0.03
2 min after treatment	2.87 ± 0.29	1.97 ± 0.16	0.72 ± 0.08
4 min after treatment	4.52 ± 0.41	12.63 ± 1.04	6.11 ± 0.07
8 min after treatment	5.39 ± 0.52	42.30 ± 2.97	9.80 ± 1.06
10 min after treatment	1.95 ± 0.20	21.32 ± 1.94	7.41 ± 0.79

Note. Results are mean values ± SD of three experiments. Kallikrein activity is in each case expressed as kallikrein equivalent to ng of bradykinin/mg of protein, determined by bioassay in cell fractions obtained from half of pooled kidneys of 10 loaded rats.

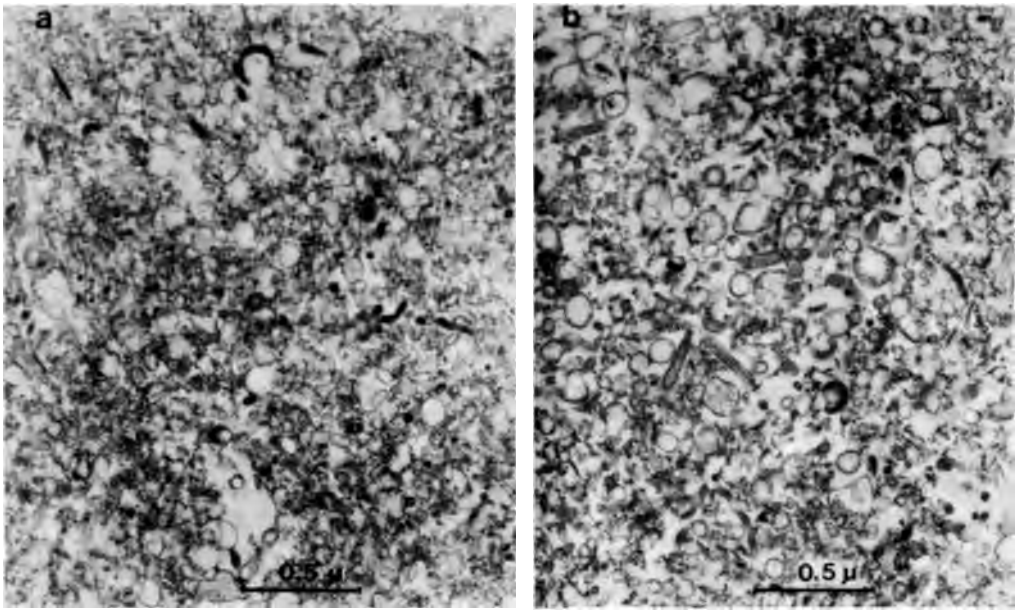
TABLE II. DISTRIBUTION OF RENAL KALLIKREIN ACTIVITY IN KIDNEY SUBCELLULAR FRACTIONS OBTAINED FROM SODIUM-LOADED RATS

	Salt loaded		Salt loaded and colchicine			
			0.5 mg/100 g body wt		1.0 mg/100 g body wt	
	Total protein (mg)	Enzyme activity	Total protein (mg)	Enzyme activity	Total protein (mg)	Enzyme activity
Homogenate	763.8	4.52	614.6	4.31	694.1	4.47
1000*g* pellet	339.4	5.69	286.2	5.88	293.4	5.68
Light crude mitochondrial fraction	78.9	12.63	60.2	3.61	79.9	1.92
Lysosomes	10.1	0.14	5.8	0.15	7.6	0.15
Microsomes	61.1	6.11	60.5	10.11	59.2	18.72
Supernatant	248.3	0.28	211.9	0.01	231.6	0.05

Note. Values are in each case the average of three experiments obtained in cell fractions from half the kidney pool of 10 salt-loaded rats, after 4 min of treatment. Kallikrein activity, measured by bioassay, is expressed as in Table 1. Supernatant is the fraction of the homogenate which does not sediment at 100,000*g* after 1 hr.

treatment with two doses of colchicine and salt loading for 4 min, in the distribution of kallikrein activity in different fractions. The highest activities were found in the microsomal fractions which increased nearly two- (0.5 mg colchicine) and threefold (1.0 mg colchicine). The inverse situation is observed in rats not treated with colchicine, which show a higher activity in the light crude mitochondrial fraction than in the microsomal fraction. Since the composition of a subcellular fraction depends on the method of isolation, it should be indicated that the light crude mitochondrial fraction as well as the microsomal fraction, isolated by differential centrifugation, are mixed fractions. Thus, in addition to mitochondria, the light crude mitochondrial fraction includes lysosomes, granules, and heavy microsomes. On the other hand, the microsomal fraction is mostly composed of fragmented rough and smooth endoplasmic reticulum, including membranes of the Golgi complex. As shown in Table II, in sodium-loaded rats, the pretreatment with colchicine induces an increase of activity in the microsomal fraction and a decrease in the light crude mitochondrial fraction. In spite of the different kallikrein content, the protein distribution in the different fractions is only slightly affected, even in rats treated with a high dose of colchicine. Furthermore, the low kallikrein activity in lysosomes, isolated from the light crude mitochondrial fraction, remains unaffected by the colchicine treatment (see Fig. 3).

Figures 1 and 2 illustrate the morphology of the microsomal and the light crude mitochondrial fractions obtained from sodium-loaded rats treated and not treated with 1.0 mg of colchicine, sacrificed 4 min after treatment with salt. A similar and typical morphology in both microsomal fractions is observed, consisting essentially of closed and empty vesicles, ruptured vesicles, membrane fragments, and vesicles marked by ribosomes attached to their membrane. On the other hand, the difference in morphology between both mitochondrial fractions is quite clear. Although both are rich in mitochondria, the fraction obtained from kidneys of rats not treated with colchicine shows a larger number of dense bodies than the one obtained from colchicine-treated rats. Since similar activity of acid phosphatase was found in both fractions, we suggest that the difference in morphology observed is due to the presence of granules of secretion or secretory vesicles. Thus, the light crude mitochondrial fraction of colchicine-nontreated rats seems to be richer in granules or vesicles



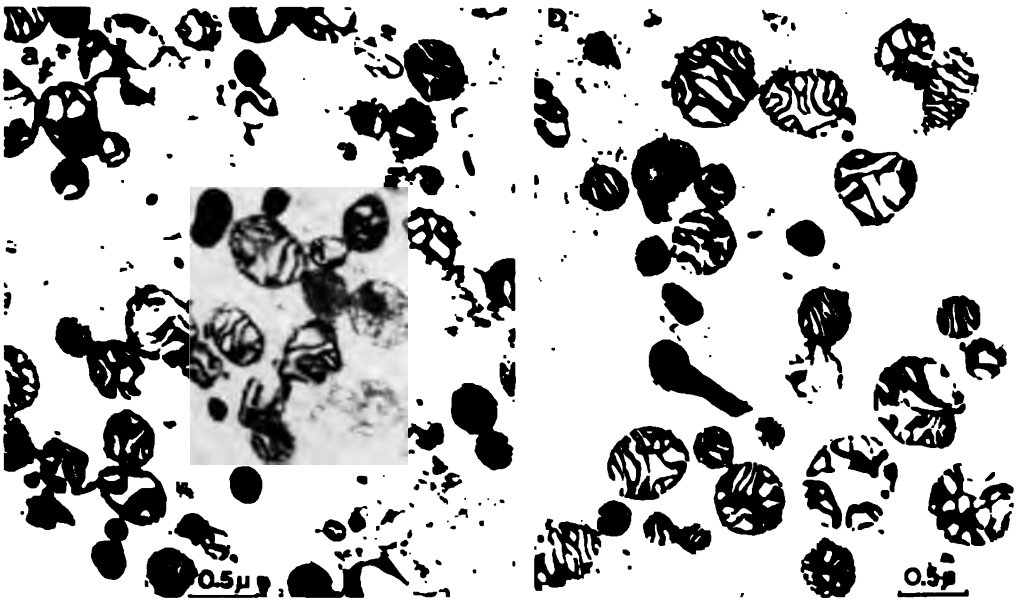


FIG. 1. Electron micrograph of a microsomal fraction obtained from sodium-loaded rat kidney homogenate. (a) Untreated with colchicine ×16,480; (b) treated with colchicine, ×16,480.

FIG. 2. Electron micrograph of a light crude mitochondrial fraction obtained from kidney homogenate of sodium-loaded rat. (a) Untreated with colchicine, ×17,600; (b) treated with colchicine ×17,600.

than the mitochondrial fraction of colchicine-treated rats.

Table III illustrates the kallikrein activity of the microsomal fraction and its components, obtained from pooled kidneys of 10 rats, treated with 1.0 mg of colchicine, and killed 4 min after sodium loading. Since most kallikrein activity resides in micro-

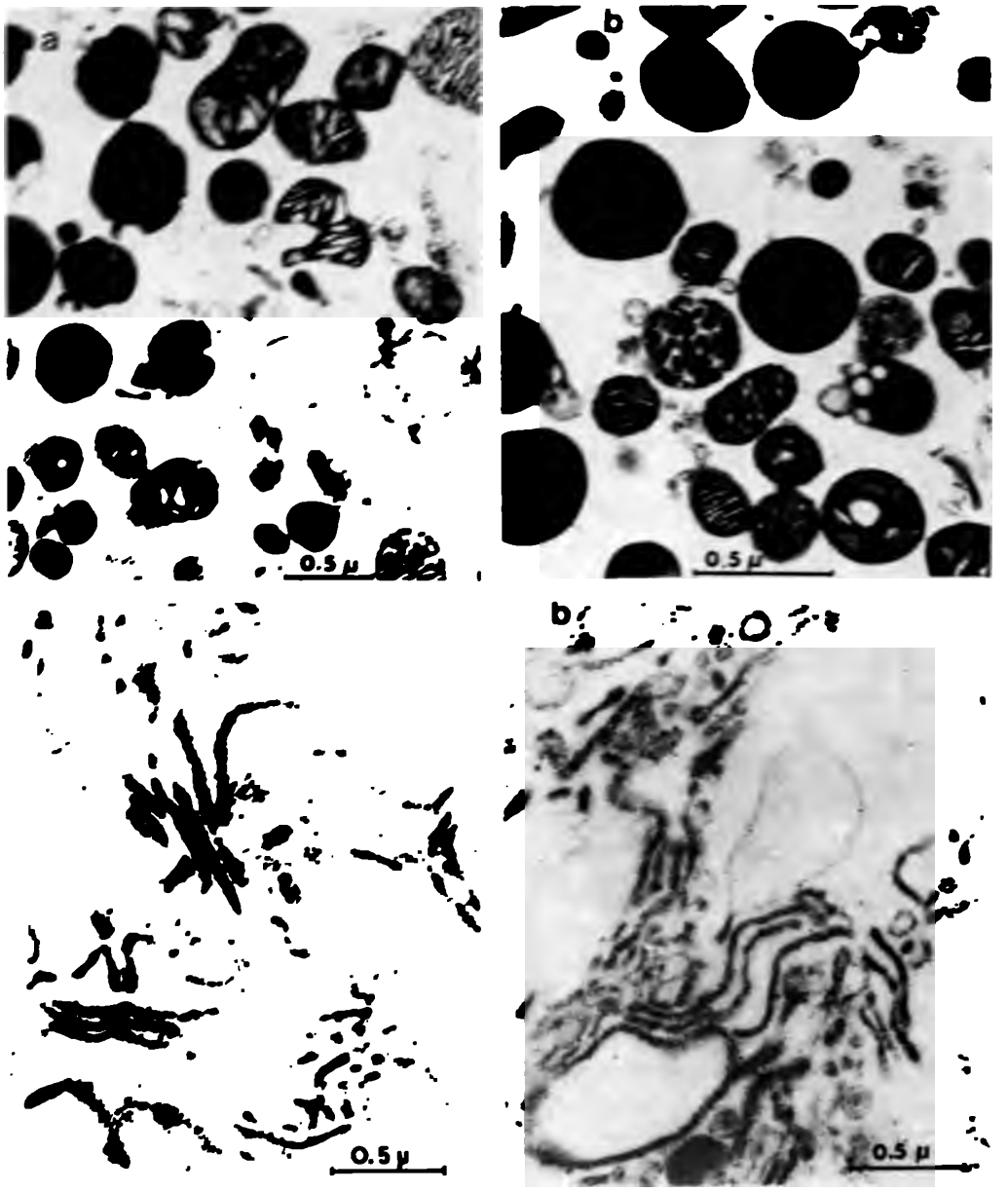


FIG. 3. Electron micrograph of a lysosomal fraction obtained from the light crude mitochondrial fraction of Fig. 2. (a) Untreated with colchicine, ×18,400; (b) treated with colchicine, ×18,400.

FIG. 4. Electron micrograph of a Golgi complex-rich fraction (equivalent to fraction 1 + 2) obtained by step sucrose gradient of sodium loaded rat kidney homogenate. (a) Untreated with colchicine, ×16,200; (b) treated with colchicine, ×16,800.

somes (see Table II), the enzyme was measured in total microsomes as well as in microsomes divided into smooth and rough fractions, obtained from half the pooled kidneys. Kallikrein activity was also measured in the Golgi complex-rich fractions 1 and 2, obtained from the remaining half of the pooled kidneys. The kallikrein activity in

TABLE III. RENAL KALLIKREIN ACTIVITY OF DIFFERENT COMPONENTS OF THE MICROSOMAL FRACTION OF SALT-LOADED COLCHICINE-TREATED RATS

	Colchicine (1.0 mg/100 g wt)	
	Total protein (mg)	Enzyme activity
Homogenate	672.0	4.36
Microsomes	54.45	18.83
Rough microsomes	39.94	2.90
Smooth microsomes	10.74	70.24
Golgi complex-rich fraction 1	1.74	134.26
Golgi complex-rich fraction 2	2.79	175.35
Supernatant	220.42	0.04

Note. Fractions were isolated from the same pool of kidneys from 10 treated rats. Values are the mean of three experiments and are expressed as in Table I. Total microsomes, smooth and rough microsomes, and the supernatant were obtained from half the pooled kidneys. The remaining tissue was used to isolate Golgi complex-rich fractions. The protein content of each fraction is referred to as the whole homogenate.

the smooth microsome fraction was almost 24-fold higher than that in the rough microsome fraction. The activity in the smooth microsome fraction was almost four times higher than that in the total microsomes. The smooth microsome fraction (Table V) shows a galactosyltransferase activity which indicates a 12% of contamination with Golgi complex, from the transferase activity found in Golgi complex-rich fraction 2 (Table V). Golgi complex-rich fractions 1 and 2 have the highest kallikrein activity. The combined Golgi complex fractions contain 71 and 96% of the kallikrein found in total microsomes and in smooth microsome fractions, respectively.

TABLE IV. DISTRIBUTION OF RENAL ESTERASE ACTIVITY IN DIFFERENT COMPONENTS OF THE MICROSOMAL FRACTION OF SALT-LOADED COLCHICINE-TREATED RATS

Fraction	Esterase activity
Homogenate	0.145 ± 0.016
Microsomes	0.280 ± 0.019
Rough microsomes	0.160 ± 0.014
Smooth microsomes	0.970 ± 0.010
Golgi complex-rich fraction 1	1.800 ± 0.150
Golgi complex-rich fraction 2	1.905 ± 0.186
Supernatant	0.025 ± 0.003

Note. Values represent the mean ± SD of three preparations obtained from 10 pooled, colchicine-pretreated, and sodium-loaded rat kidneys. Enzyme activity is expressed as μmole BAEE hydrolyzed/min/mg of protein, at 37°.

In addition, the stimulating effect of the different fractions on uterine contractility were 100% sensitive to trasylol.

A similar distribution of activities in all the mentioned fractions was obtained when the activity of esterase was determined, as is shown in Table IV. Again, in animals previously treated with colchicine, the two Golgi complex fractions show the highest level of kallikrein activity.

Since the Golgi complex-rich fractions have low yields of proteins, both assays used to measure activity of kallikrein were not sufficiently sensitive to measure it in Golgi complex-rich fractions of nonloaded rats, in the absence of an enzymatic induction. For this reason we could not measure it in these fractions.

Since the amount of protein in the Golgi complex fractions 1 and 2 was rather low, both fractions were combined. The morphologies are shown in Fig. 4, and indicate that they derive predominantly from the Golgi complex. The Golgi complex fraction from colchicine-treated rats seems to contain more loaded Golgi complex elements.

As shown in Table V, the Golgi complex-rich fractions 1 and 2, isolated from sodium-loaded rat kidneys, were enzymatically unique compared to other purified cell fractions (see Fig. 5). Thus, these fractions exhibit the highest level of activity for galactosyltransferase, and appear

TABLE V. DISTRIBUTION OF "MARKER ENZYMES" IN PURIFIED SUBCELLULAR FRACTIONS OF SODIUM-LOADED RAT KIDNEYS

Fractions	Total protein (mg)	Amount of phosphorus per mg protein (μg)	Succinate cytochrome c	Glucose-6-phosphatase	Acid phosphatase	Galactosyl transferase[a]
Homogenate	763.8	13.40	0.145	0.029	0.564	11.92
Light mitochondria	49.8	10.42	0.753	0.012	0.604	N.D.
Lysosomes	10.1	13.02	0.290	0.051	2.143	N.D.
Smooth microsomes	12.2	28.17	0.018	0.275	0.343	38.16
Rough microsomes	43.4	34.29	0.024	0.253	0.101	8.70
Golgi-rich fraction 1	2.3	32.64	0.027	0.059	0.061	235.29
Golgi-rich fraction 2	4.4	30.64	0.010	0.041	0.166	312.19
Supernatant	250.2	13.69	0.000	0.022	0.281	N.D.[b]

Note. Values are the mean of three preparations from five pooled rat kidneys, obtained after 4 min of salt loading.

[a] Nanomole hr mg of protein at 37°; all other activities expressed as μmole/min/mg of protein at 37°, except glucose-6-phosphatase which was carried out at 37°.

[b] Not detected.

to be about 70 and 75% pure, respectively.

The glucose-6-phosphatase activity present in smooth microsomes shows that the Golgi complex fractions contain about 15 to 20% endoplasmic reticulum contamination. The acid phosphatase activity indicates that they are contaminated 3 to 8% with lysosomes. Succinate cytochrome *c* reductase activity suggests that they are also contaminated (1 to 4%) with mitochondria. From the galactosyltransferase activity found in Golgi complex-rich fraction 2, it appears that smooth and the total microsomal fractions are contaminated with the Golgi complex, about 9 and 12%, respectively. On the other hand, the level of acid phosphatase activity in the light mitochondrial fraction appears to indicate a 28% contamination with lysosomes.

The same marker enzymes, in purified subcellular fractions of sodium-loaded rats and sodium-loaded rats pretreated with low and high doses of colchicine were measured, and the activities did not differ significantly from the values presented in Table V. In general, the values illustrated in this table are quite similar to the specific activity of the same marker enzymes of purified organelles isolated from untreated or from unloaded rat kidneys (29).

Discussion. Due to previous evidence suggesting different sites of subcellular localization of kallikrein biosynthesis and secretion we focused our efforts on localizing the organelles possibly involved in the biosynthesis and/or activation of renal kallikrein. In order to analyze the conflicting evidence available on the subject, we have isolated different subcellular fractions from kidneys of rats submitted to two different treatments. Our purpose was to inhibit the secretion process and to obtain an increase in renal kallikrein content.

As we reported previously (11) acute NaCl loading induces in the rat a rapid and considerable increase of renal kallikrein that could be due to the existence of a sodium receptor in the gastrointestinal tract or to a rapid change in the release mechanism of renal kallikrein following sodium absorption.

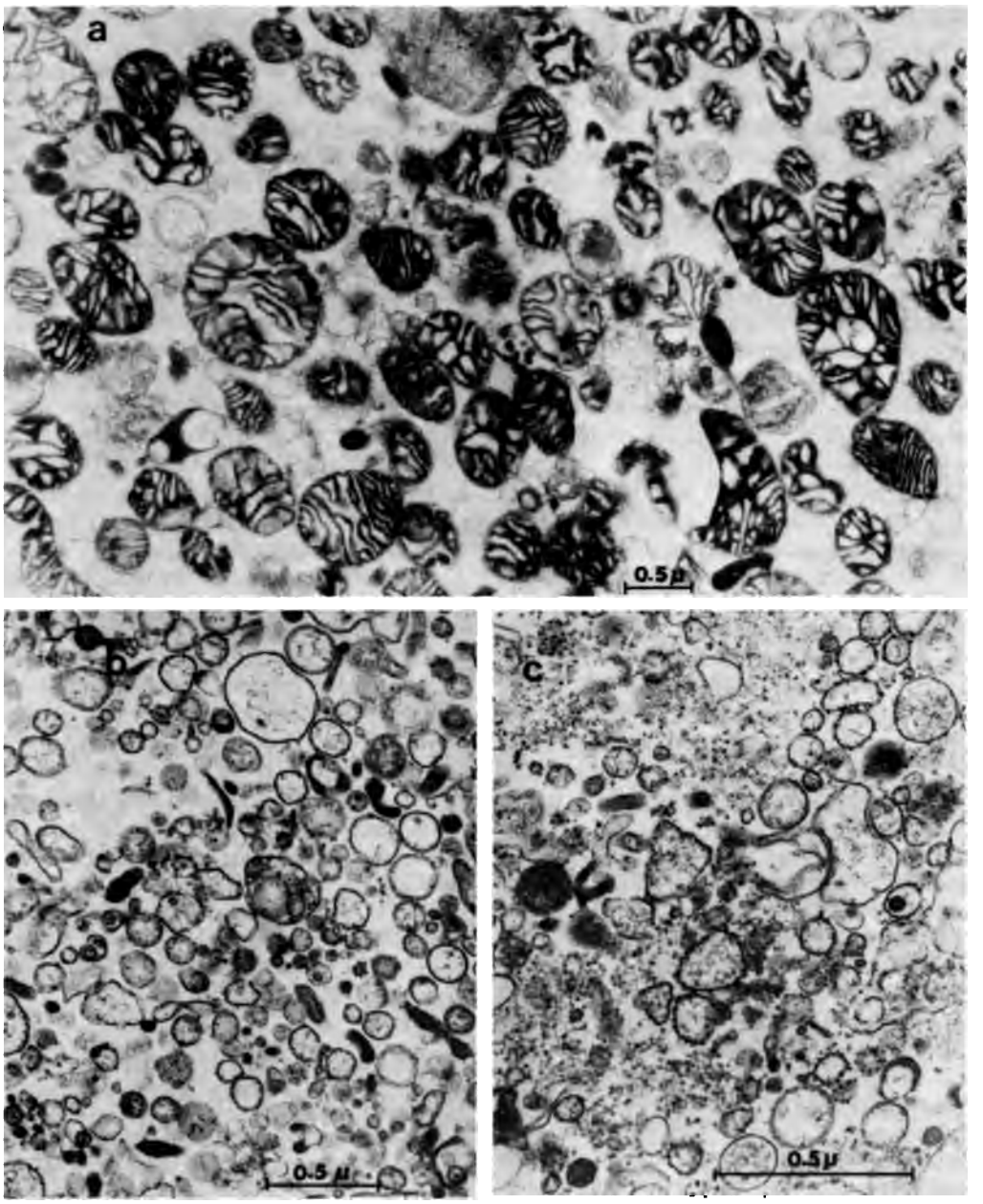


FIG. 5. Electron micrograph of a purified fraction obtained by fractionation of a kidney homogenate from a sodium-loaded rat. (a) Mitochondria-rich fraction, ×19,200; (b) smooth microsome-rich fraction, ×25,600; (c) rough microsome-rich fraction, ×29,600.

Our present results demonstrate that subcellular fractions, isolated by differential centrifugation from sodium-loaded animals not treated with colchicine (Table I), have a high activity of kallikrein in the crude light mitochondrial fraction. This fraction has a significant lysosomal contamination as is indicated by its acid phos-

phatase activity (Table IV). Based on the phosphatase activity, our results agree with those obtained by Carvalho and Diniz (6) and Baggio *et al.* (7). These authors postulated that lysosomes are the site where most of the kallikrein activity resides. However, results obtained by Chiang *et al.* (30) and Geipert and Erdös (5) suggest that kallikrein is secreted into the extracellular space in the form of dense granules and probably through fusion with the plasma membrane. Our studies seem to indicate that this mechanism may also occur in kidney cells. When using colchicine, a secretion inhibitor that does not interfere with the protein transport from the rough endoplasmic reticulum to the Golgi complex (13), the enzyme activity was recovered in the microsomal fraction, specifically in the Golgi complex-rich fraction. This indicates that the action of colchicine at the Golgi complex level may affect the secretion of granules containing kallikrein. Moreover, the acid phosphatase activity remains unaffected by colchicine treatment, indicating that lysosomal contamination in the light crude mitochondrial fraction of nontreated and colchicine-treated rats was similar. The fact that high kallikrein activity appears in the microsomal fraction after colchicine treatment is in agreement with results obtained by Nustad and Rubin (9). These authors have reported that kallikrein is mainly localized in this membranous fraction. Microsomes, isolated according to our procedure (29), mostly include fragmented rough and smooth endoplasmic reticulum as well as Golgi complex membrane. For this reason, we associate the site of biosynthesis and/or activation of kallikrein with the Golgi complex.

Studies by Redman (13) have shown that colchicine, administered to whole rat, blocks the secretion of serum albumin in rat liver cells blocking the release of this component from the Golgi complex. Our data suggest that colchicine administered to whole animal also blocks the release of kallikrein from this organelle in kidney cells. Carvalho and Diniz (6) and Baggio *et al.* (7) reported a high level of kallikrein activity in the lysosomal fraction. We suggest that their results can be explained by the fact that kallikrein is secreted as secretory granules, which are physicochemically similar to the kidney lysosomes, and that after homogenation both sediment together.

Renal kallikrein is a glycoprotein with a yet not determined carbohydrate sequence. Nevertheless, it is clear that glycoproteins are synthesized in the rough endoplasmic reticulum (peptidic backbone), and then transported to the Golgi complex (31). In this last organelle, sugars are added stepwise to the nonreducing end of the carbohydrate chains of the glycoprotein, by the specific action of galactosyl or syalyl transferase, using nucleotide sugar glycosyl as donors (32). It is most likely that kallikrein is not only concentrated but also modified in the Golgi complex by the addition of a terminal sugar.

The evidence offered in this paper supports the idea that kallikrein is transported within renal cells by a system of secretory vesicles derived from the Golgi complex. Our data also suggest that lysosomes may not play an important role in this process. On the other hand, since our data indicate that kallikrein activity is present in microsomes, and particularly in the Golgi complex, the biosynthesis and/or activation of renal kallikrein seems to occur in these organelles.

Finally, our results provide a feasible explanation for the apparently conflicting results on the sites of renal kallikrein biosynthesis and/or activation.

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1. Webster ME, Gilmore JP. Influence of kallikin-10 on renal function. Amer J Physiol 206:714–718, 1964.
2. Carretero OA, Scicli AG. Renal killikrein its localization and possible role in renal function. Fed Proc 35:194–198, 1976.
3. Ørstavik TB, Nustad K, Brandtzaeg P, Pierce JV.

Cellular origen of urinary kallikrein. H. Histochem Cytochem 24:1037–1039, 1976.
4. Nustad K, Vaaje J, Pierce JV. Synthesis of kallikrein by rats kidney slices. Brit J Pharmacol 53:229–234, 1975.
5. Geipert F, Erdos EG. Properties of granule that contain kallikrein and renin. Experientia 27:912–913, 1971.
6. Carvalho IF, Diniz GR. Kining forming enzyme (kininogenin) in homogenate of rat kidney. Biochim Biophys Acta 128:136–148, 1966.
7. Baggio B, Favaro S, Antonello A, Zen F, Borsatti A. Subcellular localization of renin and kallikrein in rat kidney. Ital J Biochem 24:199–206, 1975.
8. Nustad K. Localization of kininogenase in the rat kidney. Brit J Pharmacol 39:87–89, 1970.
9. Nustad K, Rubin I. Subcellular localization of renin and kininogenase in the rat kidney. Brit J Pharmacol 40:326–333, 1970.
10. Ward PE, Erdos EG, Gedney CD, Dowben RM, Reynolds RC. Isolation of renal membranes that contain kallikrein, angiotensin 1-converting enzyme (kininase (II) and angiotensinase in the rat. Clin Sci Mol Med 51:267s–270s, 1976.
11. Rojas M, Brandan E, Zambrano F. Salt loading effect on renal kallikrein activity in rat. Proc Soc Exp Biol Med 163:151–154, 1980.
12. Reaven E, Reaven G. Evidence that microtubules play a permissive role, in hepatocyte very low density lipoprotein secretion. J Cell Biol 84:28–29, 1980.
13. Redman CM, Bernerjee D, Howell D, Palade GE. Colchicine inhibition of plasma protein release from rat hepatocytes. J Cell Biol 66:42–59, 1975.
14. Barnafi L, Rosas R, de la Lastra M, Croxatto HR. Influence of oxitocin and vasopresion on sodium-retaining activity of aldosterone in the rat. Amer J Physiol 198:255–259, 1960.
15. Fleischer B, Zambrano F. Golgi apparatus of rat kidney. Preparation and role in sulfatide formation. J Biol Chem 249:5995–6003, 1974.
16. Stain Y, Widnell C, Stein O. Acylation of lysophosphatides by plasma membrane fractions of rat liver. J Cell Biol 39:185–192, 1968.
17. Fleischer S, Kervina M. Subcellular fractionation of rat liver. In: Fleischer S, Packer L, eds. Methods in Enzymology. New York, Academic Press, Vol. 31:pp6–41, 1974.
18. Maunsbach AB. Isolation of kidney lisosomes. In: Fleischer S, Packer L, eds. Methods in Enzymology. New York, Academic Press, vol. 31:pp330–339, 1974.
19. Dallner GP, Siekevitz P, Palade GE. Biogenesis of endoplasmic reticulum membranes in developing rat hepatocyte. J Cell Biol 30:97–115, 1966.
20. Swanson MA. Glucose-6-phosphatase from liver. In: Colowick S, Kaplan NO, eds. Methods in Enzymology. New York, Academic Press, Vol. 2:pp541–543, 195.
21. Fleischer S, Fleischer B. Removal and binding of polar lipids in mitochondria and other membrane systems. In: Stabrook RW, Pullman ME, eds. Methods in Enzymology. New York, Academic Press, Vol. 10:pp406–433, 1967.
22. Besseys OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J Biol Chem 164:321–326, 1946.
23. Chen PS, Toribara TY, Warner H. Microdetermination of phosphorus. Anal Chem 28:1756–1758, 1976.
24. Fleischer B. Isolation and characterization of Golgi apparatus and membrane from rat liver. In: Fleischer S, Packer L, eds. Methods in Enzymology. New York, Academic Press, Vol. 31:pp180–191, 1974.
25. Croxatto HR, Noe E. Kallikrein-like enzyme in purified renal extracts containing renin. Comentat Pontif Acad Sci 40:1–2, 1971.
26. Brown ME. The colorimetric determination of arginine ester hydrolysis by human sera. J Lab Clin Med 55:616–624, 1960.
27. Lowry OL, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
28. Fleischer B, Fleischer S, Osawa H. Isolation and characterization of Golgi membranes from bovine liver. J Cell Biol 43:59–79, 1969.
29. Fleischer B, Zambrano F, Fleischer S. Biochemical characterization of the Golgi complex of mammalian cells. J Supramol Struct 2:737–750, 1974.
30. Chiang TS, Erdos EG, Miwa IM, Tague LL, Coalson J. Isolation from salivary gland of granules containing renin and kallikrein. Cir Res 23:507–517, 1968.
31. Morre J, Outracht L. Dynamics of the Golgi apparatus. Membrane differentiation and membrane flow. Int Rev Cytol (Suppl 5):61–188, 1977.
32. Fleischer B. The role of Golgi apparatus in the synthesis of glicoproteins and glycolipids. In: Harmon RE, ed. Cell Surface Carbohydrate Chemistry. New York, Academic Press, Vol. 1:pp27–47, 1978.

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Erratum

Volume 171, No. 4 (1982) in the article, "Annual Report of the Executive Secretary and Editor for the Year Ending December 31, 1981," pages 523–524, on page 524, the following lines were omitted and are to replace lines 10 and 11:

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17β-Hydroxysteroid Dehydrogenase Activity in Tissues of Fetal Rhesus Macaques (41503)

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Abstract. 17β-Hydroxysteroid dehydrogenase (17β-HSDH) activity was measured in tissues (anterior pituitary, diencephalon, frontal cortex, liver, whole blood, and uterus) of fetal rhesus macaques on Days 80, 120, and 150 of gestation. The production of estrone (E_1) (quantified by radioimmunoassay) by an 800*g* supernatant incubated with excess substrate (estradiol-17β [E_2]) was used as an index of 17β-HSDH activity. Over a 30-min incubation period E_1 was produced in a linear fashion by all the tissues that we studied. The pH optimum for this activity in the pituitary gland was ~9. Boiling of the tissue for 1 min reduced its activity significantly. Significant changes in 17β-HSDH activity during development were observed for two of the six tissues studied. Activity in fetal anterior pituitary glands was greatest on Day 80 of gestation but changed to significantly lower levels by Day 120 ($P < 0.01$). This low level continued on Day 150 of gestation. The pattern of activity in the liver was different from that in the pituitary gland. High activity was found on Day 80, and this increased significantly by Day 120 ($P < 0.05$). The levels of activity observed on Day 150 were similar to those found on Day 120. Low levels of 17β-HSDH activity were found in the central nervous system, whole blood, and uterus. In rhesus adults as in fetuses we have found relatively high levels of 17β-HSDH activity in the anterior pituitary gland. The biological significance of changing levels of this activity during gestation is not known.

Many tissues of the reproductive system are estrogen sensitive, but the roles of metabolic enzymes in mediating estrogen action are not well understood. We do know, however, that estrogens such as estradiol-17β (E_2) can be converted to weaker compounds such as estrone (E_1) by microsomal enzymes, the 17β-hydroxysteroid dehydrogenases (17β-HSDHs), in reproductive tissues (1). Estrone is less active than E_2, apparently because it is less competitive for the estrogen receptor (2) and diffuses out of target cells more easily (3). Considering these two differences alone in the properties of E_1 and E_2, one can envision effective modulation of the actions of E_2 by its cellular conversion to E_1. In uterine tissue from women, the 17β-HSDHs are regulated by progesterone and the activity of these enzymes varies with the stage of the reproductive cycle (3). Similar observations have been made in uteri (4) and pituitaries (5) from rhesus macaques, but the significance of these observations is not well understood. All of the above studies involved tissues from adult animals. Recently, we had the opportunity to obtain tissues from fetal monkeys at different times in gestation, and we determined the activity of the 17β-HSDHs in these tissues.

Materials and Methods. *Animals and tissue preparations.* Tissues were obtained from 12 rhesus macaque (*Macaca mulatta*) fetuses of known gestational ages (Days 80, 120, and 150) that had been delivered by cesarean section. The three Day-80 and the three Day-150 fetuses were intact females. The six Day-120 fetuses comprised both males and females; some of these had been gonadectomized 3 weeks before and given testosterone or saline infusions for 6 hr. In this latter group we combined the data from the various treatment groups because no differences appeared evident from the following comparisons. The 17β-HSDH activity in anterior pituitary glands of two

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female fetuses spayed *in utero* on Day 100 of gestation and treated with testosterone for 21 days ranged from 220 to 238 ng E_1 mg protein^{-1} after a 30 min incubation. Pituitaries from four males similarly treated contained 207–363 (range) ng E_1 mg protein^{-1}. In a 15-min incubation a pituitary gland of a female not treated with testosterone contained 132 ng E_1 mg protein^{-1} whereas the activities of this enzyme in the pituitaries of two females treated with testosterone were 130 and 154 ng E_1 mg protein^{-1}, respectively. These preliminary observations revealed no striking differences between treatments thereby providing the rationale for combining animals between treatments. At delivery the fetuses received cold saline infusions to remove blood from tissues to be analyzed. The brains and pituitaries were quickly removed and chilled with ice. Under these conditions the various tissues were dissected, weighed, placed in ice-cold buffer, and homogenized. The following tissues were removed and analyzed for 17β-HSDH activity: liver, uterus, anterior pituitary, frontal cortex, and diencephalon (a small block of tissue between the optic chiasm and the mammillary bodies about 2 mm thick). Each tissue was homogenized in 1 ml of ice-cold tris(hydroxymethyl)aminomethane (Tris) buffer, pH 8.0, at 37°C in a 5-ml glass homogenizer. The homogenate was poured into a 13 × 100-mm glass tube and centrifuged at 800*g* in a Sorvall refrigerated centrifuge. An aliquot of the supernatant was removed for Lowry protein determinations. An aliquot of 1.5 mg of protein from the 800*g* supernatant was incubated in 0.75 ml Tris buffer with different quantities of E_2 and with a 10-fold molar excess of NAD for different periods of time. Each incubation flask contained 1.5 mg of protein, an amount we had already demonstrated to be on the linear portion of the activity versus protein concentration curve.

Chromatography. After incubation the steroid was extracted from the incubation medium in 6 ml of ether. The ether extract was taken to dryness under a stream of air in a water bath at 37°C. The samples, dissolved in a small volume of chloroform: methanol (1:1, v/v), were applied to the origins of paper strips with capillary tubes. We used Whatman No. 1 paper strips (55 cm long and 2.5 cm wide) that had been cleaned by being boiled in methanol. The chromatograms were developed in benzene: formamide. Benzene was the mobile phase, and the paper was soaked in formamide: acetone (1:1, v/v). In this system it takes approximately 3 h for the solvent front to reach the end of the paper. E_2 and E_1 (R_fs of 0.33 and 0.71, respectively) clearly separate in this chromatography system). After chromatography, the paper strips were dried at room temperature for 16 hr. Standard solutions of E_1 and E_2 fractionated with the samples on individual paper strips were used as references for elution. The standards were visualized on paper by immersion of the paper strips in Barton's reagent, i.e., 1% $K_3Fe(Cu)_6$ and 1% $FeCl_3 \cdot 6H_2O$ (1:1, v/v) (6). An area with the mobility of E_1 was eluted with 10 ml of methanol from each paper chromatogram. The methanol extract was taken to dryness and dissolved in 1 ml of ethanol. One-tenth of the ethanol extract was dried, dissolved in 100 μl of hexane: benzene:methanol (62:20:13, v/v), applied to a Sephadex LH-20 column, and fractionated in the same solvent system mentioned above. The E_1 area was collected from the Sephadex columns and quantified by radioimmunoassay (RIA). We estimated procedural losses by adding 10,000 cpm of [^{3}H]estrone (E_1) to duplicate samples which we carried through the entire procedure. Using these independent estimates of recovery, we adjusted the final quantity of E_1 in each sample for procedural losses. Although E_1 was quantified by an antiserum that cross-reacts with E_2, we achieved specificity in our E_1 assay by chromatography on paper and Sephadex LH-20 columns. This was demonstrated by the low blanks for E_1 (<10 pg) when no tissue, only substrate, was added. These blanks were subtracted in the process of computing the E_1 values. The measurement of E_1 was used as an index of 17β-HSDH activity.

Statistics. Differences between the mean

production of E_1 among gestational ages were analyzed by *t* tests after the homogeneity of variance had been determined.

Results. In a previous publication we validated our use of RIA measurements of E_1 as an assay for 17β-HSDH activity in tissues from adult rhesus macaques (5). Similar information on fetal tissues is presented in Figures 1 through 3. Assay of the E_1 produced from E_2 by the 800*g* supernatant of fetal pituitaries and liver revealed a linear response up to minute 30 of incubation, the last time period mentioned in Fig. 1. Measurements of E_1 in the 800*g* supernatant at time 0 revealed small amounts of E_1 in the tissue before the incubation had begun. These amounts were subtracted from the 15- and 30-min values; and from all other data presented in this manuscript. Similar responses for other tissues such as uterus, cortex, and whole blood were obtained, but less E_1 was formed by these tissues *in vitro*. Figure 2 demonstrates the effect of increasing substrate concentrations on 17β-HSDH activity after a 15-min incubation. It appears that 80 μM E_2 the limit of solubility in our buffer is a near saturating quantity of substrate. The effects of pH and heat treatment (boiling for 1 min) are shown in Fig. 3. The pH maximum was about pH 9.0 since the activity declined rapidly at pH 10.0. These data generated for liver may or may not be applicable to other tissues. Boiling the 800*g* supernatant for 1 min

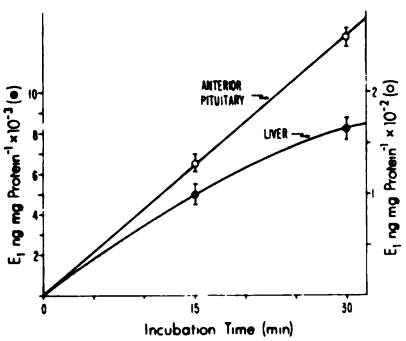

FIG. 1. Effects of incubation time on 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity of liver and anterior pituitary glands from Day-120 fetuses (n = 6).

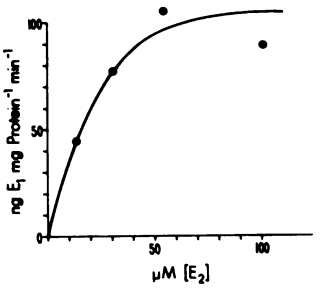

FIG. 2. Effects of substrate concentration (estradiol-17β [E_2]) on 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity in Day-120 fetal pituitaries.

drastically reduced the capacity of the tissue to convert $E_2 \rightarrow E_1$ but some activity remained. This activity was not subtracted from any of the values reported in this manuscript.

We used this technique to analyze the 17β-HSDH activity in various tissues from fetuses as a function of stage of gestation (Fig. 4). The greatest activities occurred in fetal livers and anterior pituitary glands. Activity levels in other tissues were much lower. Pituitary glands from Day-80 fetuses contained significantly more enzyme activity than pituitary tissues taken at later times in gestation ($P < 0.01$). Activity in liver, however, increased significantly from Day 80 to Day 120 of gestation ($P < 0.05$). Activities of all other tissues were low and did not seem to change as gestation progressed.

Discussion. An understanding of the function of metabolic enzymes for promoting hormone action in target tissues is beginning to emerge. For target tissues such

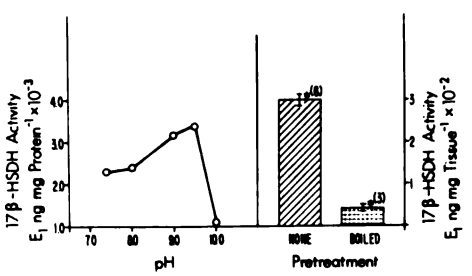

FIG. 3. Effects of pH and heat treatment on 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity in liver from Day-120 fetuses.

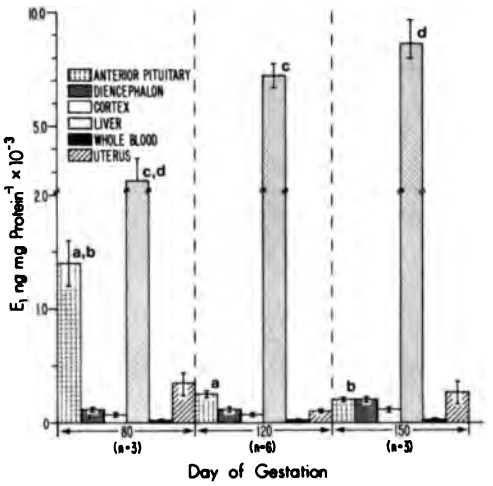

FIG. 4. Effects of age on 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity in fetal tissues from rhesus macaques. Tissues were incubated with 80 μM E_2 and an excess of NAD for 30 min. We used the amount of E_1 produced by this extract as an index of 17β-HSDH activity. E_1 was quantified by a specific RIA after chromatography on paper and LH-20 columns. See Methods section for more details about procedures. Bars with the same letters differed significantly ($P < 0.01$ for a, b and $P < 0.05$ for c, d).

as prostate (7) and perineal skin (8), production of dihydrotestosterone (DHT) from testosterone appears to be an essential conversion for androgen action. Likewise, the aromatization of testosterone to E_2 in neural tissue appears to be important in many species for sexual differentiation of the brain and negative feedback control of gonadotropin secretion by steroids (9, 10). The 17β-HSDHs are found in a number of mammalian tissues such as erythrocytes (11), uterine endometrium (1), liver (12), and placenta (13). The amounts of 17β-HSDH activity in human endometrium increase during the secretory phase of the cycle and in response to exogenous progesterone (14). Similar results have been obtained in uterine tissue from rhesus macaques (4). In that study both E_2 and progesterone appeared to increase the activity of these enzymes. In addition to those in the uterus, we found high levels of 17β-HSDH activity in adult anterior pituitary glands (5). Data on fetal pituitaries reported in this paper confirm this fact, but only with respect to fetuses in early gestation. Some reports link the production of DHT and E_2 from testosterone to the cellular action of this hormone in certain tissues, but the significance of the biological activity of the 17β-HSDHs is more difficult to understand. In uterine endometrium from rhesus macaques the favored conversion is $E_2 \rightarrow E_1$ rather than vice versa (4). The production of E_1 from E_2 can be viewed as simply a breakdown process or it can be construed as a more significant and dynamic way of controlling E_2 levels within the target cell. If one takes the latter point of view, the effectiveness of E_2 in initiating its biological action is proportional to the amount of active hormone that binds to the estrogen receptor. Therefore, study of the production and control of 17β-HSDH activity seems important to an understanding of the way in which E_2 acts on target cells.

The connection between the presence of significant amounts of 17β-HSDH activity and a significant biological function is the unknown factor at this time. There is good evidence in some rodent species that aromatization of androgen to estrogen is important for sexual differentiation of the fetus. Our measurements of E_2 in the serum of the fetus have shown very low or undetectable quantities of this hormone early in gestation, when differentiation of the nervous system and anlagen of the reproductive tract occurs (15). Concentrations of E_2 in the maternal serum, however, were relatively high. Apparently, the primate fetus develops a mechanism to maintain low levels of E_2 within itself and thus prevent abnormalities in sexual differentiation. The question is: Do 17β-HSDH activities in the liver and pituitary have a significant role in this process? There are analogous situations in which the metabolic conversion of steroid hormones correlates with physiological effects previously unexplained. Doves castrated several weeks earlier are insensitive to androgens that mediate perch calling (16). This insensitivity correlates with an increase in 5β-reductase activity in the preoptic region of the brain (16). There are precedents in the literature for the idea that enzymes involved in the metabolism of

steroids to inactive products serve important biological functions. The fact that their activities can be regulated by compounds such as progesterone or E_2 and the fact that they can be affected by gonadectomy indicate their importance in the physiological regulation of cells.

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1. Tseng L, Gurpide E. Estradiol and 20α-dihydroprogesterone dehydrogenase activities in human endometrium during the menstrual cycle. Endocrinology 94:419–423, 1974.
2. Tseng L, Gurpide E. Effect of estrone and progesterone on the nuclear uptake of estradiol by slices of human endometrium. Endocrinology 93:245–248, 1973.
3. Tseng L, Gurpide E. Changes in the *in vitro* metabolism of estradiol by human endometrium during the menstrual cycle. Amer J Obstet Gynecol 114:1002–1108, 1972.
4. Kreitman O, Kreitman-Gimbal B, Hodgen GD. 17-β-Hydroxysteroid dehydrogenase in monkey endometrium: Characterization of enzyme activity and effects of estradiol alone or in combination with progesterone. Steroids 34(6):693–703, 1979.
5. Resko JA, Stadelman HL, Norman RL. 17β-Hydroxysteroid dehydrogenase activity in the pituitary gland and neural tissue of rhesus monkeys. J Steroid Biochem 11:1429–1434, 1979.
6. Barton GM, Evans RS, Gardner JAF. Paper chromatography of phenolic substances. Nature (London) 170:249–250, 1952.
7. Wilson JD, Gloyna RE. The intranuclear metabolism of testosterone in the accessory organs of reproduction. Recent Prog Horm Res 26:309–336, 1970.
8. Northcutt RE, Island DP, Little GW. An explanation for the target organ unresponsiveness to testosterone in the testicular feminization syndrome. J Clin Endocrinol Metab 29:422–425, 1969.
9. Mainwaring WIP. The mechanism of action of androgen. Monogr Endocrinol, No. 10, New York, Springer Verlag, p35, 1977.
10. Resko JA, Quadri SK, Spies HG. Negative feedback control of gonadotropins in male rhesus monkeys: Effects of time after castration and interactions of testosterone and estradiol-17β. Endocrinology 101:215–224, 1977.
11. Migeon CJ, Lescure WH, Zinkham WH, Sidbury JB Jr. *In vitro* interconversion of 16-C^{14}-estrone and 16-C^{14}-estradiol-17β by erythrocytes from normal subjects and from subjects with a deficiency of red-cell glucose-6-phosphate dehydrogenase activity. J Clin Invest 41:2025–2053, 1962.
12. Baird DT. Synthesis and secretion of steroid hormones by the ovary *in vivo*. In: Zuckerman S, Weir BJ, eds. The Ovary. New York, Academic Press, Vol III:p305, 1977.
13. Engel LL, Groman EV. Human placental 17β-estradiol dehydrogenase: Characterization and structural studies. Recent Prog Horm Res 30:139–169, 1974.
14. Tseng L, Gurpide E. Induction of human endometrial estradiol dehydrogenation by progestins. Endocrinology 97:825–833, 1975.
15. Resko JA, Ploem JG, Stadelman HL. Estrogen in fetal and maternal plasma of the rhesus monkey. Endocrinology 97:425–430, 1975.
16. Hutchison JB, Steiner T. Brain 5β-reductase: A correlate of behavioral sensitivity to androgen. Science 213:244–246, 1981.

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Responsiveness of Cerebral Osmoreceptors in the Anesthetized Dog (41504)

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Abstract. Experiments were carried out to determine if, in the anesthetized dog undergoing a water diuresis, selective elevation of cerebral osmolality induced an antidiuresis. Bilateral intracarotid infusion of hypertonic sodium chloride in an amount previously shown to increase jugular plasma osmolality by 3% was associated with a significant decline in C_{H_2O}. The same volume of hypertonic sodium chloride infused intravenously did not alter C_{H_2O} significantly. These experiments do not support the view that anesthesia blocks the response of cerebral osmoreceptors.

The concept that cerebral osmoreceptors modulated the secretion of antidiuretic hormone (ADH) was suggested by Verney as the result of studies done in the 1940s (1). He observed that intracarotid bolus injections of hypertonic NaCl inhibited a water diuresis in the conscious dog. Since the antidiuretic response could be mimicked by the injection of posterior pituitary extract, Verney suggested that elevation of cerebral osmolality caused shrinkage of osmoreceptor cells which, in turn, led to the release of ADH.

These studies were not seriously challenged until the work of Bie. He found that in anesthetized dogs, elevation of cerebral osmolality by an estimated 5% had no effect on a water diuresis (2). In addition, the time course and extent of the antidiuresis produced by an ic or iv 60-min infusion of a hypertonic NaCl solution was the same. Although at this time Bie did not deny the possibility that Verney was correct, he questioned if the response observed by Verney was specific.

Bie has provided an in-depth exhaustive review of the concept of osmoreceptors, in which he reaffirms his position that the stimuli used in osmoreception studies have not been shown to be physiological osmostimuli (3). However, a very important part of the review is provided as an addendum in which a study by Dietz *et al.* (4) is discussed. This group observed that in the conscious dog, a bilateral ic, 10-min infusion of hypertonic NaCl that was shown to increase jugular osmolality by only 3%, inhibited a water diuresis. Since Dietz *et al.* used conscious dogs and Bie anesthetized dogs, Bie suggested that his failure to obtain results consistent with a central osmoreceptor hypothesis was due to the fact that he used anesthetized dogs while Verney and Dietz *et al.* used conscious dogs, i.e., anesthesia blocks the cerebral osmoreceptor mechanism. To test this hypothesis, we carried out experiments in a group of anesthetized dogs, including those used by Dietz *et al.*, to determine if, when anesthetized with the same anesthesia used by Bie, they would show an antidiuresis when cerebral osmolality is elevated within a physiologic range. Contrary to Bie's hypothesis, the results show that anesthesia does not block the cerebral osmoreceptor mechanism.

Methods. The study was conducted using a group of female American Foxhounds weighing between 20 and 27 kg which had been prepared with bilateral carotid loops. The dogs were fasted for 18 hr before an experiment.

They were anesthetized with 60 mg/kg α-chloralose (Sigma Chemical) and 10

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mg/kg sodium pentobarbital (Nembutal, Abbott) administered via an intracath placed in a saphenous vein and advanced to the level of the right heart. A Foley catheter was placed in the bladder *via* the urethra and the carotids were catheterized with 2-in., 18-gauge angiocaths. Blood pressure and heart rate were monitored via one carotid line except during the bilateral IC infusion.

A steady state water diuresis was established by infusing a volume of hypotonic solution of 25 m*M* urea and 40 m*M* glucose equivalent to 2% of body weight over a period of one hour. To maintain the diuresis, the urine output in any given period was matched by infusing an equal amount of glucose–urea solution.

When a steady state urine flow rate was achieved, a 10-min, bilateral ic or a 10-min iv infusion of hypertonic NaCl was performed. The NaCl solution was infused at a rate of 45 μM/kg body weight/min/artery or 90 μM/kg/min intravenously. Blood samples for electrolytes, osmolality, creatinine concentration, and, in some experiments, arginine vasopressin (ADH) radioimmunoassay (6) were taken immediately before and 9 min into the infusion period, and again at 60 min after the hypertonic infusion. At the end of the experiments, the animals were returned to their kennel to recover from the anesthesia.

Data were analyzed using one-way analysis of variance for repeated measures. Significance reported is $P < 0.05$.

Results. In five dogs given 90 μM/min/kg NaCl intravenously (Fig. 1B), no significant change occurred from the mean control creatinine clearance (GFR) of 52 ml/min. Free water clearance (C_{H_2O}) (control mean = 2.2 ml/min), urinary sodium excretion (UNa$\dot{V}$) (control = 13.7 μeq/min), and urinary potassium excretion (UK$\dot{V}$) (control = 8.1 μeq/min) did not change significantly from control values. Heart rate (HR) and blood pressure (BP) did not change. Plasma sodium concentration increased from a control of 141 meq/liter to 143 meq/liter after the hypertonic NaCl infusion and remained elevated for the duration of the experiment. Plasma osmolality increased from 290 to 294 mOsm/liter. In the three experiments in which it was measured,

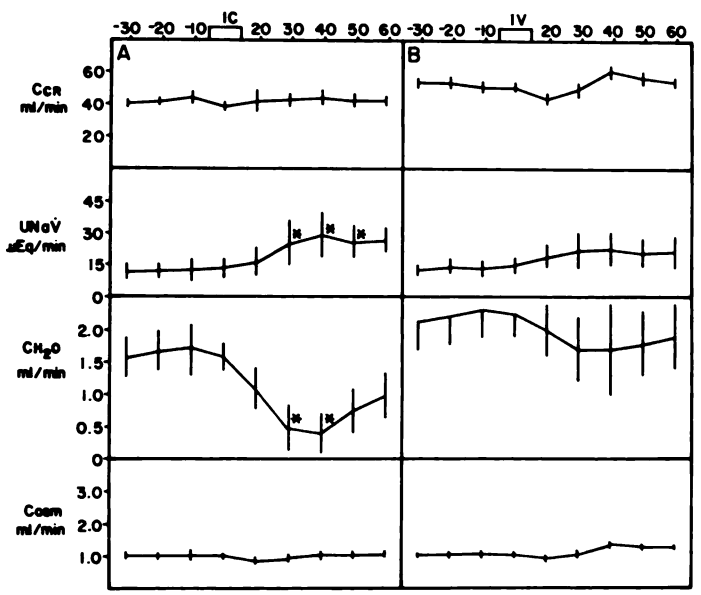

FIG. 1. Results of ic (Panel A) and iv (Panel B) infusions of hypertonic NaCl solution. C_{cr}, creatinine clearance; UNa$\dot{V}$, urinary sodium excretion; C_{H_2O}, free water clearance; C_{osm}, urinary osmolar clearance. Asterisks indicate statistical significance, $P < 0.05$.

ADH concentration remained less than 1.25 pg/ml.

The response to bilateral ic infusion of hypertonic NaCl in eight dogs is shown in Fig. 1A. Creatinine clearance remained near the control value of 42 ml/min throughout the experiments. UNa$\dot{V}$ increased significantly by 30 min after the beginning of the ic infusion, to a peak value of 28.8 μeq/min from a control level of 12.5 μeq/min. C_{H_2O} decreased significantly by 30 min after the beginning of the ic infusion from a control level of 1.7 ml/min to a minimum of 0.4 ml/min indicating a pronounced antidiuretic effect of the ic infusion. No significant change occurred from a control UK$\dot{V}$ of 11.3 μeq/min, or in HR or BP. Plasma sodium concentration showed precisely the same increase as with the iv infusion, that is, from 141 meq/liter to 143 meq/liter. Plasma osmolality increased from 288 to 292 mOsm/liter. In the six experiments in which it was measured, ADH concentration remained less than 1.25 pg/ml.

Discussion. The results clearly show that selective increases in cerebral osmolality in the chloralose anesthetized dog is associated with a significant antidiuresis. These results are consistent with the results of Verney (1) and Dietz *et al.* (4) in the conscious dog but inconsistent with the results of Bie (2, 3) in the anesthetized dog. Bie infused 83 μM/kg/min ic, bilaterally for 8 min and observed an increase in free water despite the fact that by the end of the infusion, peripheral venous osmolality had increased by approximately 7 mOsm/kg. The increase (4 mOsm/liter) in peripheral osmolality in the present experiments caused by an ic infusion was associated with an antidiuresis. However, the peripheral plasma osmolality in Bie's experiments was 283.6 mOsm/liter while in the present experiments, it was 290 (iv) and 288 (ic) prior to the hypertonic sodium chloride infusion.

Using dehydration as a stimulus in conscious dogs, Robertson (5) found that the threshold P_{osm} for ADH release was 286 mOsm/liter while we found under these conditions a value of 297 mOsm/liter (Huffman and Gilmore, unpublished observations). Studies in man and rats indicate that under conditions of hypervolemia (as in the present experiments and those of Bie due to the induction of a H_2O diuresis), the osmotic threshold for ADH release would be increased. It is, therefore, quite possible that in the Bie experiments, because of the low resting P_{osm} (283.6), the hypertonic NaCl infusions did not cause P_{osm} to reach the osmotic threshold for ADH release while in the present experiments, because of the higher P_{osm}, threshold was reached and thus an antidiuresis ensued. The reason why the resting P_{osm} in Bie's experiments in which he infused hypertonic NaCl for 8 min was lower than in the present experiments is probably because he produced a 4% body weight hydration while we produced a 2% body weight hydration. Again, this greater state of hydration (hypervolemia) would be expected to produce a greater increase in the osmotic threshold for ADH release. The diuresis observed by Bie might be explained as the result of increased fluid delivery to distal nephron sites resulting in an increased generation of C_{H_2O}.

A major obstacle to conclusive results in studies such as these is the difficulty in measuring changes in ADH. Because of the hydration, even the relatively moderate 2% of body weight used in the present study, ADH is suppressed below concentrations at which changes from steady state, control concentrations can be measured accurately. From the ADH data reported under Results, it can be concluded that the 2% hydration was sufficient to suppress ADH release in response to the hydration. Whether any increase in ADH occurred due to ic or iv hypertonic NaCl infusion cannot be determined; it can only be stated that any increase was not sufficient to raise plasma ADH above 1.25 pg/ml, the lower limit of the assay used (6).

In summary, the present study demonstrates the responsiveness of cerebral osmoreceptors during chloralose/pentobarbital anesthesia, supporting the cerebral osmoreceptor concept of Verney (1).

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1. Verney EB. The antidiuretic hormone and the factors which determine its release. Proc R Soc London Ser B 135:25, 1947–1948.
2. Bie P. Studies of cerebral osmoreceptors in anesthetized dogs: The effect of intravenous and intracarotid infusion of hyper-osmolar sodium chloride solutions during sustained water diuresis. Acta Physiol Scand 96:306, 1976.
3. Bie P. Osmoreceptors, vasopressin, and control of renal water excretion. Physiol Rev 60:961, 1980.
4. Dietz HR, Bie P, Gilmore JP, Share L, Zucker IH. The relation between carotid solute concentration and renal water excretion in conscious dogs. Acta Physiol Scand 114:45, 1982.
5. Robertson GL, Athar S, Shelton RL. Osmotic control of vasopressin function. In: Andreoli TE, Grantham JJ, Recto FC Jr. eds. Disturbances in Body Fluid Osmolality. Bethesda, Md, Amer Physiol Soc, p130, 1977.
6. Cornish KG, Gilmore JP. Increased left atrial pressure does not alter renal function in the conscious primate. Amer J Physiol (Reg Integrative Comp Physiol 12), R119–R124, 1982.

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Age- and Strain-Related Differences in Metabolic Response to Tyramine in Rats (41505)

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Abstract. Differences in metabolic response to intraperitoneally injected tyramine were examined in young (3–4 month) and old (20–24 month) Sprague–Dawley and Fischer 344 rats. Fischer 344 rats were found to be significantly more sensitive to tyramine than Sprague–Dawley. Optimal doses were 2 mg/kg body weight for young F344, 20 mg/kg for young Sprague–Dawley, 5 mg/kg for old F344, and >40 mg/kg for old Sprague–Dawley. Significantly higher doses were required by old rats than young. Mechanisms of these age- and strain-related differences in response are discussed. This information could well be important in selecting appropriate ages and strains of animals for experimental use, especially in aging research.

Tyramine, a naturally occurring sympathomimetic amine, has been identified as a normal constituent of some mammalian tissues (1). Exogenously administered tyramine is actively taken up by the sympathetic nerve endings into the catecholamine storage vesicles displacing catecholamines, primarily norepinephrine (NE), in the process (2). There are no apparent signs of toxicity when tyramine is employed, and the elevated metabolism following injections of either tyramine or NE is equivalent (3). In environmental physiological research tyramine has supplanted the use of NE as an indicator of the level of cold acclimation (3–6).

There is some information suggesting that the function of the adrenosympathetic nervous system is modified consequent to the aging process (7–11). There are two primary questions: (a) do older animals respond differently to equivalent doses of a chemical substance than younger animals; and (b) are there differences in response between various strains of a species? Heroux *et al.* (3) reported that the optimal dose of tyramine to elicit a maximum metabolic response in young male Sprague–Dawley (S–D) rats was 20 mg/kg body weight intraperitoneally. It is of practical interest to determine whether this optimal dose of tyramine for young animals is optimal for older (2 years old) animals. Available data revealed that male Sprague–Dawley rats have a median survival rate of 23 (12), 24.5 months (13, 14), and 30 months (15), while male Fischer 344 (F344) rats have a median survival rate of 27.5 (16) to 29 months (17). There are significant differences in mean body weights between the two strains of male rats. At 80 to 90 days of age, male Sprague–Dawley had a mean weight of 325 g while F344 weighed 214 g (18). At 20 to 24 months, male Sprague–Dawley attained mean body weights of 700 g (13) while male F344 had a mean weight of 329 g (19).

There is minimal information differentiating the responses of these strains to a stimulus, either chemical or physical. Such knowledge is imperative in selecting the appropriate strain of animal for experimental use. This paper presents the differences in metabolic response to tyramine in relation to age; i.e., young and old, and between the two strains of rat commonly utilized for aging research, Sprague–Dawley and Fischer 344.

Materials and Methods. Male Sprague–Dawley and Fischer 344 rats, 3 to 4 and 20 to 24 months of age, were studied. The animals were individually caged and provided with Purina Laboratory Chow and water *ad libitum*. A 12-hr light and dark cycle was maintained. Oxygen uptake was

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continuously measured in a constant volume, closed system (Volume Meter, Med Science, Model 160) with the rat in a sealed Plexiglas chamber while immersed in a 28° water bath, and the expired CO_2 was absorbed by soda lime. Colonic and chamber temperature were measured continuously with thermistor probes and recorded on a dual-channel recorder. Before evaluating the animals' response to tyramine, O_2 uptake and colonic temperature were first measured at 28° for 30 min to establish a baseline. Each animal was then temporarily removed from the chamber and given intraperitoneal tyramine–HCl. The animal was then returned to the chamber and measurements resumed for another hour. The resulting data were plotted and the peak response to tyramine which was generally present for several minutes was calculated. At least one week elapsed between each administration of tyramine in the same animal.

The optimal dose of tyramine–HCl administered was determined by constructing a log-dose–response (LDR) curve for each of the four groups of rats. That is, successively increasing log doses of tyramine–HCl were administered to the rats, and the metabolic response was plotted against log dose until the response curve plateaued when a higher dose of tyramine–HCl was administered. The optimal dose was defined as the lowest dose of tyramine–HCl that produced a maximal response increase in O_2 uptake and colonic temperature. The significance of the differences between O_2 uptake and colonic temperature before and after administration of tyramine was determined by using paired *t* tests.

Results. The increases in O_2 uptake and colonic temperature following tyramine injections were statistically significant from each basal value in all rats and at all dosages tested ($p < 0.001$), except in the old Sprague–Dawley rats when tested with the 20 mg/kg dose. The optimal dose (20 mg/kg bw) for young Sprague–Dawley failed to elicit a significant increase in either O_2 uptake or colonic temperature in old Sprague–Dawley rats.

Young rats. Young rats respond to an injection of tyramine with increases in O_2 uptake and colonic temperature. O_2 uptake usually peaks within 10 to 20 min following the injection and then gradually subsides and returns to basal levels in an hour or so. Peak response increases with increasing dosages until an optimal dose is reached and a plateau in response occurs. Elevation of the colonic temperature follows the rise of O_2 uptake with a time lag of 5 to 10 min. The degree of elevation corresponds with increasing dosage and then plateaus when the optimal dose is reached.

In young Sprague–Dawley ($n = 9$), three dosages (5, 10, and 20 mg/kg body weight) were used and 20 mg/kg body weight was determined to be the optimal dose (Figs. 1 and 2). A higher basal metabolic rate was observed in the young Sprague–Dawley given 20 mg compared to those given 10 mg. This change was probably related to the 2-week interval between test sessions when these young rats grew rapidly and effect of age on metabolism became noticeable. Since in some rats this dose elevated colonic temperature to 40.6°, any additional rise of colonic temperature was considered to be potentially harmful, and since in additional studies on cold-acclimated animals this tyramine dose resulted in colonic temperature approaching 42°, no higher dosage than 20 mg/kg body weight was attempted in this group.

Young F344 ($n = 13$) were initially tested with 5, 10, and 20 mg/kg body weight dosages. No obvious differences in their response could be discerned. Therefore, another group of young F344 ($n = 13$) were reevaluated at lower dosage levels (0.5, 1, and 2 mg/kg body weight). The optimal dose for young F344 was thus determined to be 2 mg/kg body weight (Figs. 1 and 2).

Old rats. Basal O_2 uptakes are lower in old animals, approximately 16 to 19 $ml \cdot kg^{-1} \cdot min^{-1}$, as compared to 21 to 27 $ml \cdot kg^{-1} \cdot min^{-1}$ in young animals. The old Fischer rats have higher basal colonic temperature (approximately 0.8° higher) than young Fischer. However, old animals also respond to tyramine by increases in O_2 uptake and colonic temperature. Most signifi-

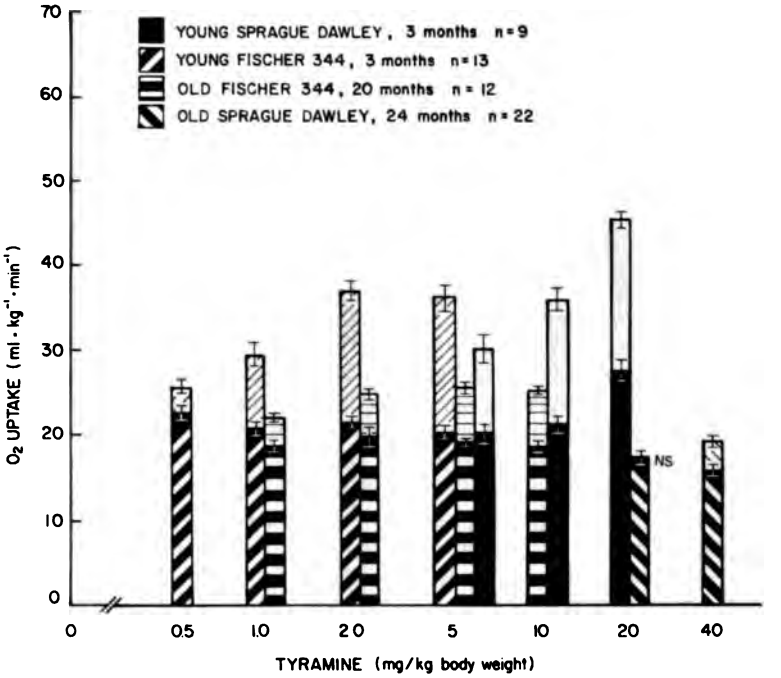

FIG. 1. Increases in oxygen uptake (clear portions of the histograms) in response to intraperitoneal injections of tyramine. All measurements were made in an ambient environment of 28°. Error bars represent SEM.

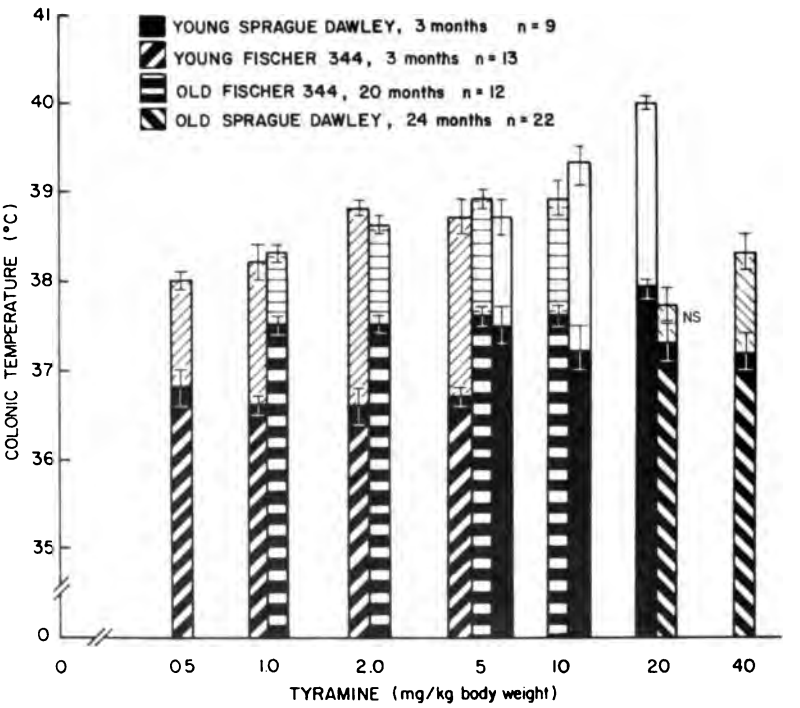

FIG. 2. Elevation of colonic temperature in response to intraperitoneal injections of tyramine. All measurements were made in an ambient environment of 28°. Error bars represent SEM.

cantly, the magnitude and time course of the response differs strikingly from the responses of the younger animals. The elevations of O_2 uptake and colonic temperature are smaller. The time course of change of O_2 uptake is similar to that of the young, but the time course for the change of colonic temperature is altered. Peaking of colonic temperatures is usually delayed 50 to 60 min. Two hours or more are required before basal levels are reattained.

The optimal dose for old F344 (n = 12) was determined to be 5 mg/kg body weight. Although the increase of O_2 uptake was similar at 2, 5, and 10 mg/kg doses, the elevation of colonic temperature apparently peaked at 5 mg and plateaued at 10 mg (Figs. 1 and 2). The optimal dose (20 mg/kg body weight) for young Sprague–Dawley did not elicit any significant response in old Sprague–Dawley (n = 5) (Figs. 1 and 2). A dose of 40 mg/kg body weight was then given and significant increases in O_2 uptake and colonic temperature ($P < 0.001, n = 22$) were obtained. No higher dosage was attempted at this point; the optimal dose for old Sprague–Dawley being apparently greater than 40 mg/kg body weight.

Discussion. The optimal doses of tyramine for each group of rats studied (2 mg/kg body weight for young F344, 5 mg/kg body weight for old F344, 20 mg/kg body weight for young Sprague–Dawley, and >40 mg/kg body weight for old Sprague–Dawley) represent a significant difference between young and old in each respective strain and between strains of either young or old animals.

The sluggish and prolonged response in old animals may be explained by altered absorption, metabolism, or excretion of drugs in old animals (20, 21). It is known that the myocardial function and blood flow to organs and tissues in old animals are reduced (22). There are functional alterations in liver and kidney (22). However, the high optimal doses found for old animals cannot be explained by these altered mechanisms alone, since old animals had been shown to be more sensitive to drugs, especially to norepinephrine (8), than young ones (21). Since tyramine acts by release of endogenous NE, this increased rather than decreased dose requirement in older animals could be due to:

(1) Decreased NE synthesis in old animals. Aging impairs the uptake of tyrosine or dopa into neurons as well as the intracellular hydroxylation of tyrosine and the β-oxidation of dopamine (7). Therefore, the available NE for release by tyramine is smaller, and more tyramine is required to produce an apparent effect. Diminished availability of NE can also be responsible for the reduced metabolic effect observed in old animals.

(2) Decreased re-uptake of NE in nerve endings in aging animals. Since tyramine is taken up at the nerve ending by the same amine-pump mechanism, the uptake of tyramine can also be slowed (7). Reduced re-uptake of NE indicates a reduced availability of NE for release by tyramine.

(3) Increased monoamine oxidase (MAO) activity in old rats. Both NE and tyramine are substrates for MAO. Increased MAO activity may result in reduced available NE and tyramine (23).

The prolonged response or decreased rate of recovery in old animals represents an example of the often observed phenomenon that the aged require more time than the young to reestablish homeostasis when displacements are induced (22). It is also of interest that we found a relatively lower basal O_2 uptake but higher basal colonic temperature in old F344 than in young F344. This may suggest more efficient heat production, less effective heat dissipation, or better insulation in older F344.

The strain-related difference observed in this study is of considerable significance since Sprague–Dawley and F344 are the two most commonly used strains in age-related studies. Mazze *et al.* (24) compared the rate of metabolism of methoxyflurane to inorganic fluoride and in its nephrotoxic effects among five rat strains: Fischer 344, Buffalo, Wistar, Sprague–Dawley, and Long–Evans. F344 rats were found to be more susceptible to inorganic fluoride than all other strains. They suggested that genetic factors were responsible for the difference. Although some other unknown fac-

tors may be responsible for the difference in response between the F344 and Sprague–Dawley rats observed in this study, we are inclined to accept the suggestion that genetic factors were responsible for the difference.

From our data it is apparent that generalizations as to the physiological alterations induced or present in aging animals cannot be made lightly. Whether or not the strain differences observed have a direct effect on aging processes remains to be determined.

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1. Tallman JF, Saavedra JM, Axelrod J. Biosynthesis and metabolism of endogenous tyramine and its normal presence in sympathetic nerves. J Pharmacol Exp Ther 199:216–221, 1976.
2. Axelrod J, Gordan E, Hertting GG, Kopin IJ, Potter LT. On the mechanism of tachyphylaxis to tyramine in the isolated rat heart. Brit J Pharmacol 19:56–63, 1962.
3. Heroux O, Pagé E, Le Blanc J, Leduc J, Gilbert R, Villemaire A, Rivest P. Nonshivering thermogenesis and cold resistance in rats under severe cold conditions. J Appl Physiol 38:436–442, 1975.
4. Kiang-Ulrich M, Horvath SM. Effects of cold acclimation and 6-OHDA sympathectomy on cold tolerance of rats. J Therm Biol 4:233–237, 1979.
5. Kiang-Ulrich M, Horvath SM. Successful cold acclimation following bilateral adreno-demedullation in rats. Proc Soc Exp Biol Med 162:449–453, 1979.
6. Kiang-Ulrich M, Horvath SM. Effects of cold acclimation and 6-OHDA sympathectomy on cold tolerance of bilaterally adrenodemedullated rats. J Therm Biol 5:179–183, 1980.
7. Gey KF, Burcard WP, Pletscher A. Variation of the norepinephrine metabolism of the rat heart with age. Gerontologia 11:1–11, 1965.
8. Frolkis VV, Bezrukov VV, Bogatshaya LN, Verkhratsky NS, Zamostian VP, Shevtchuk VG, Shtchegoleva IV. Catecholamines in the metabolism and functions regulation in aging. Gerontologia 16:129–140, 1970.
9. Freedman LS, Ohuchi T, Goldstein M, Axelrod F, Fish S, Dancis J. Changes in human serum dopamine β-hydroxylase activity with age. Nature (London) 236:310–311, 1972.
10. McGeer EG, McGeer PL. In: Neurobiology of Aging. New York. Plenum. p287. 1975.
11. Stramentinoli G, Gualano M, Catto E, Algeri S. Tissue levels of S-adenosylmethionine in aging rats. J Gerontol 32:392–394, 1977.
12. Jones DC, Kimeldorf DJ. Lifespan measurements in the male rat. J Gerontol 18:316–321, 1963.
13. Lesser GT, Deutsch S, Markofsky J. Aging in the rat—longitudinal and cross-sectional studies of body composition. Amer J Physiol 225:1472–1478, 1973.
14. Berg BN, Harmison CR. Growth, disease and aging in the rat. J Gerontol 12:370–377, 1957.
15. Adelman RC. Loss of adaptive mechanisms during aging. Fed Proc 38:1968–1971, 1979.
16. Hoffman HJ. Survival distributions for selected laboratory rat strains and stocks. DHEW Publication No. NIH 79-161, pp19–34, 1979.
17. Coleman GL, Barthold SW, Osbaldistan GW, Foster SJ, Jonas AM. Pathological changes during aging in barrier-reared Fischer 344 male rats. J Gerontol 32:258–278, 1977.
18. Sines JO. Strain differences in activity, emotionality, body weight and susceptibility to stress induced stomach lesions. J Genet Psychol 101:209–217, 1962.
19. Chesky JA, Rockstein M. Life span characteristics in the male Fischer rat. Exp Aging Res 2:399–407, 1976.
20. Kato R, Vassanelli P, Frontino G, Chiesara E. Variation in the activity of liver microsomal drug-metabolizing enzymes in rats in relation to the age. Biochem Pharmacol 13:1037–1051, 1964.
21. Richey DP, Bender AD. Pharmacokinetic consequences of aging. Annu Rev Pharmacol Toxicol 17:49–65, 1977.
22. Shock NW. In: Powers JH, ed. Surgery of the Aged and Debilitated Patient Philadelphia, Saunders, pp10–43, 1968.
23. Lai FM, Berkowitz B, Spector S. Influence of age on brain vascular and cardiovascular monoamine oxidase activity in the rat. Life Sci 22:2051–2056, 1978.
24. Mazze RI, Cousins MJ, Kosek JC. Strain differences in metabolism and susceptibility to the nephrotoxic effects of methoxyflurane in rats. J Pharmacol Exp Ther 184:481–488, 1973.

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Reserpine Inhibits Rat Anterior Pituitary Hormone Secretion *in Vitro*: Effects on GH, TSH, and LH (41506)

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Abstract. We have extended our observations on the ability of reserpine to inhibit secretion of anterior pituitary hormones *in vitro*. The release of newly synthesized [^{3}H]GH and radioimmunoassayable TSH and LH from female rat anterior hemipituitary glands was measured after 5 hr incubation in [^{3}H]leucine in the presence or absence of reserpine. Reserpine, 9 μM, blocked the release of each hormone stimulated by 50 mM K^+ but not basal hormone secretion. Stimulation of [^{3}H]GH release by 10 μM PGE_1, or 5 mM dbcAMP was not affected by 5 μM reserpine, while stimulation of TSH release by 70 nM TRH was significantly blunted with 5 μM reserpine. The influence of reserpine on pituitary hormone secretion may define a pattern suggesting that the drug interferes with utilization of the extracellular but not the intracellular calcium mobilized during the secretory process.

Reserpine is usually administered *in vivo* to effect biogenic amine depletion through blockade of aminergic reuptake mechanisms. Used in this manner, reserpine causes hyperprolactinemia indirectly through dopamine depletion and thus reduction of the inhibitory tone exerted on the lactotroph (1). Reserpine *in vitro*, however, inhibits prolactin secretion from rat anterior pituitary glands (2, 3). This unexpected effect was dose-related (between 0.09 and 9 μM) and apparently independent of the traditional aminergic interactions of reserpine (4). The present study was designed to explore further the effects of reserpine *in vitro* on the secretion of other pituitary hormones. We have investigated basal and stimulated secretion of newly synthesized ^{3}H-labeled growth hormone ([^{3}H]GH) and radioimmunoassayable LH and TSH from female rat anterior pituitary glands in the presence or absence of reserpine.

Methods. Hormone secretion was evaluated by established techniques (5). Briefly, anterior pituitary glands from adult female Sprague–Dawley rats of 200–220 g (Dominion Laboratories, Dublin, Va.) were removed and bisected prior to 1000 hr. Three hemipituitaries from three different rats were pooled, weighed, and placed into an incubation flask containing 1 ml of tissue culture medium M-199 (M.A. Bioproducts, Walkersville, Md.) or Earle's MEM (GIBCO Labs, Grand Island, N.Y.) and 10 μCi [^{3}H]leucine (40–50 Ci/mmole, Amersham, Arlington Heights, Ill.). Each experimental group had four flasks. The flasks were then incubated for 5 hr under 95% O_2–5% CO_2 on a Dubnoff shaker at 37°.

Samples of incubation medium were assayed for [^{3}H]GH, TSH, and LH. Secretion of newly synthesized [^{3}H]GH was measured by polyacrylamide gel electrophoresis and liquid scintillation spectrometry with the incorporated radioactivity expressed relative to anterior pituitary wet weight as counts per minute [^{3}H]GH per milligram pituitary (5). Standard double antibody radioimmunoassay was used to measure TSH and LH with reagents kindly supplied by Dr. A. F. Parlow and the NIAMDD. These results were expressed as micrograms hormone per milligram pituitary relative to the reference preparations TSH RP-1 and LH RP-1. The intraassay coefficients of variation for TSH and LH were 10 and 9.8%, respectively.

Experimental agents were added to the

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[illegible]

[illegible]

[illegible]

hormone is secreted under these conditions remains unknown.

Secretion of ³H GH from rat pituitary glands was also stimulated by [illegible] PGE₂ or [illegible] dibutyryl cAMP in two separate experiments (Table II). Unlike the K⁺-stimulated ³H GH release, reserpine did not influence ³H GH secretion by these two agents.

The stimulatory effect of TRH on TSH secretion was evaluated in the presence or absence of reserpine (Fig. 1). Release of TSH was stimulated by [illegible] TRH (P < 0.01 vs control) but in the presence of [illegible] reserpine the TRH action was reduced (P < 0.01 vs TRH alone).

Discussion. Previously we observed that under in vitro conditions reserpine inhibited secretion of newly synthesized prolactin and speculated that the drug altered cellular calcium kinetics to achieve this effect (3, 4). Now we report that reserpine selectively inhibits the secretion of other pituitary hormones under a variety of specific conditions. Stimuli such as K⁺, PGE₂, dbcAMP, and TRH each have selective effects on the secretion of pituitary hormones and they produce their enhanced hormone release through specific mechanisms.

Extracellular calcium is required for both the K⁺-stimulated release of hormones and neurotransmitters (5) and pituitary produc-

[illegible] Release of ³H GH, TSH, and LH [illegible]

[illegible]	³H GH [illegible]	TSH (μg/mg pituitary)	LH (μg/mg pituitary)
	Hormone released into the incubation medium		
[illegible]	[illegible]	[illegible]	[illegible] ± 0.02
[illegible]	[illegible]	[illegible]	0.71 ± 0.12
[illegible]	[illegible]	36.03 ± 2.34	2.61 ± 0.18
[illegible]	[illegible]	[illegible]	1.46 ± 0.07

[illegible] flasks containing Medium 199 to which reserpine [illegible] glands taken from six rats. Each value represents the hormone [illegible] the mean [illegible] for the group. Newly synthesized ³H GH was measured [illegible] and liquid scintillation techniques, while TSH and LH were quantitated [illegible]

[illegible] compared to the respective control group

[illegible] compared to the respective control group

[illegible] compared to the respective K⁺ group

[illegible] compared to the respective K⁺ group

TABLE II. THE EFFECT OF RESERPINE ON [^{3}H]GH SECRETION CAUSED BY PGE_1 OR DIBUTYRYL cAMP (dbcAMP)

Treatment groups	[^{3}H]GH released into the incubation medium (cpm/mg pituitary)
Experiment 1	
Control	222 ± 50
10 μM PGE_1	734 ± 88[a]
10 μM PGE_1 + 5 μM reserpine	609 ± 46[b]
Experiment 2	
Control	800 ± 129
5 mM dbcAMP	2555 ± 191[a]
5 mM dbcAMP + 5 μM reserpine	2114 ± 346[b]

Note. The incubation parameters were as described in Table 1.
[a] $P < 0.01$ compared to the respective control.
[b] $P > 0.05$ compared to treatment without reserpine.

tin secretion (7). The present findings show that reserpine caused a partial to complete blockade of the K^+-mediated stimulation of [^{3}H]GH, TSH, and LH (Table I), and in previous studies the compound inhibited prolactin secretion (2, 3). Using other systems reserpine blocked the release of [^{3}H]DA from rat striatum (8) and norepinephrine from rabbit heart (9). These interactions suggest that reserpine may prevent effective utilization of extracellular calcium.

Stimulation of GH secretion by PGE_1 and dbcAMP was not influenced by reserpine (Table II). The action of these two secretagogues, unlike K^+-mediated secretion, is associated with intracellular calcium mobilization but not influx of extracellular calcium (10–12).

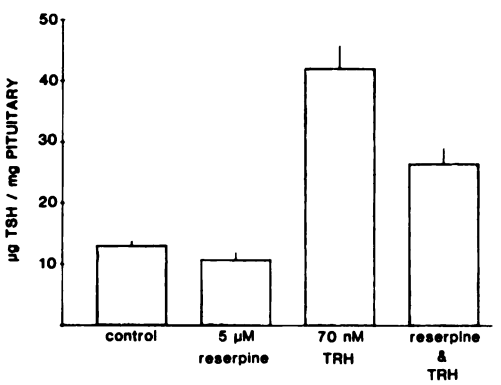

FIG. 1. The effect of reserpine on TRH-stimulated secretion of TSH *in vitro*. This experiment included eight flasks in each group, and the ordinate represents μg TSH/mg pituitary as the mean ± SE. Reserpine reduced the stimulatory effect of TRH on TSH release ($P < 0.01$ vs TRH alone).

Stimulation of TSH secretion by TRH may depend on both transport of extracellular and mobilization of membrane-bound intracellular calcium (13, 14). We found that during a 5-hr incubation the TRH effect was significantly but only partially inhibited by reserpine (Fig. 1).

Hormone secretion in the presence of reserpine seems to define a pattern from which some inferences may be drawn, although we have no direct information on the mechanism(s) involved. It is unlikely that reserpine is altering membrane properties in a general manner to inhibit secretion since (1) the effects are selective and (2) hormone release can still be stimulated by certain secretagogues. The inhibitory action of reserpine is most pronounced in those secretory events which are strongly dependent on extracellular calcium, such as basal prolactin and K^+-mediated hormone secretion. Reserpine is much less active with secretory events dependent largely on intracellular calcium. Studies are in progress to evaluate the hypothesis that reserpine interrupts calcium uptake in its action to inhibit hormone secretion.

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1. MacLeod RM, Fontham EH, Lehmeyer JE. Prolactin and growth hormone production as influenced by catecholamines and agents that affect brain catecholamines. Neuroendocrinology 6:283–294, 1970.
2. MacLeod RM. Influence of norepinephrine and catecholamine-depleting agents on the synthesis and release of prolactin and growth hormone. Endocrinology 85:916–923, 1969.
3. Login IS, MacLeod RM. The direct effect of reserpine in vitro on prolactin release from rat anterior pituitary glands. Brain Res 204:79–85, 1981.
4. Login IS, Thorner MO, MacLeod RM. Mechanisms of release of pituitary prolactin as directly influenced by reserpine. Trans Amer Neurol Assoc 105:189–191, 1980.
5. Lamberts SWJ, MacLeod RM. Studies on the effect of cyproheptadine on growth hormone secretion. Proc Soc Exp Biol Med 162:116–120, 1979.
6. Douglas WW, Poisner AM. Stimulus-secretion coupling in a neurosecretory organ: The role of calcium in the release of vasopressin from the neurohypophysis. J Physiol 172:1–18, 1964.
7. Thorner MO, Hackett JT, Murad F, MacLeod RM. Calcium rather than cyclic AMP as the physiological intracellular regulator of prolactin release. Neuroendocrinology 31:390–402, 1980.
8. Dyck LE, Boulton AA. The effect of reserpine and various monoamine oxidase inhibitors on the uptake and release of tritiated meta-tyramine, para-tyramine and dopamine in rat striatal slices. Res Commun Psychol Psychiatry Behav 5:61–78, 1980.
9. Misu Y, Kubo T, Nishio H. Acute partial sympathetic blockade by reserpine in the isolated rabbits' hearts. Eur J Pharmacol 19:267–275, 1972.
10. Eto S, Wood JM, Hutchins M, Fleischer N. Pituitary $^{45}Ca^{++}$ uptake and release of ACTH, GH and TSH: Effect of verapamil. Amer J Physiol 226:1315–1320, 1974.
11. Kraicer J, Spence JW. Release of GH from purified somatotrophs: Use of high K^+ and the ionophore A23187 to elucidate interrelations among Ca^{++}, cAMP and somatostatin. Endocrinology 108:651–657, 1981.
12. Milligan JV, Kraicer J. ^{45}Ca uptake during the *in vitro* release of hormones from the rat adenohypophysis. Endocrinology 89:766–773, 1971.
13. Morley JE. Neuroendocrine control of thyrotropin secretion. Endocrinol Rev 2:396–436, 1981.
14. Geras E, Rebecchi MJ, Gershengorn MC. Evidence that stimulation of thyrotropin and prolactin secretion by thyrotropin-releasing hormone occur via different calcium-mediated mechanisms: Studies with verapamil. Endocrinology 110:901–906, 1982.

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6-Keto-PGF$_{1\alpha}$ Synthesis in Diabetic Rat Aorta: Effect of Substrate Concentration and Cholesterol Feeding (41507)

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Abstract. The effect of short-term feeding of a 1% cholesterol diet to normal and streptozotocin-induced diabetic rats on aortic 6-keto-PGF$_{1\alpha}$ synthesis from exogenous and endogenous arachidonic acid (AA) was investigated. Diabetes and cholesterol feeding (by themselves) resulted in a reduction in aortic synthesis of 6-keto-PGF$_{1\alpha}$ from both exogenous and endogenous arachidonic acid. An additive effect of diabetes and cholesterol feeding together was found for synthesis of 6-keto-PGF$_{1\alpha}$ from exogenous but not endogenous AA. Experiments in which the AA concentration was varied suggested that the inhibition of aortic 6-keto-PGF$_{1\alpha}$ synthesis by diabetes was competitive in nature. The diabetic rat was also found to be severely compromised in its ability to handle dietary cholesterol, as evidenced by a dramatic increase in plasma total cholesterol.

Epidemiological studies have suggested that patients with diabetes mellitus have an increased susceptibility to atherosclerosis (1, 2). Abnormalities of platelet function such as increased sensitivity to ADP, epinephrine, collagen, and arachidonic acid-induced aggregation have been noted (3, 4). This is of importance since altered platelet function may contribute to the atherosclerotic process.

Platelets from diabetic patients appear to synthesize greater amounts of the proaggregatory thromboxane A_2 (TXA_2) (5–8). Aortic tissue of human diabetics (9), streptozotocin-induced (10, 11), and spontaneously (12) diabetic rats show a reduced production of the antiaggregatory prostacyclin (PGI_2) as measured by its stable breakdown product 6-keto-PGF$_{1\alpha}$ or bioassay. It has been suggested that the balance between these two prostaglandin-like substances (TXA_2/PGI_2) may be an important determinant in thrombotic disorders of diabetics (11).

Little is known about the mechanism of the decreased aortic PGI_2 synthesis in diabetes. Gerrard *et al.* (11) demonstrated that release of the substrate arachidonic acid from phospholipids was not impaired in the aorta of streptozotocin-induced diabetic rats. Rather, the defect appeared to be in the conversion of free arachidonic acid to 6-keto-PGF$_{1\alpha}$, although the reason for this defect was not determined. Furthermore, how diabetic rat aorta will respond to a stress such as cholesterol feeding, in terms of 6-keto-PGF$_{1\alpha}$ formation, is not known.

In this communication, we have attempted (a) to study the kinetics of 6-keto-PGF$_{1\alpha}$ formation from exogenous arachidonic acid in control and diabetic rat aortas and (b) to assess the sensitivity of normal and diabetic rat aorta to the short-term feeding of a 1% cholesterol diet, in terms of 6-keto-PGF$_{1\alpha}$ formation from both exogenous and endogenous arachidonic acid.

Materials and Methods. *Animals.* Randomly selected male Sprague–Dawley rats (Harlan Animal Supplies, Indianapolis, Ind.), initially weighing between 250 and 300 g, were made diabetic by intravenous (tail vein) injection of 50 mg/kg body weight streptozotocin (Sigma Chemical Co., St. Louis, Mo.) in citrate buffer (0.1 *M* citric acid and 0.145 *M* NaCl, pH 4.5). Controls were injected with an equivalent volume of citrate buffer. The induction of diabetes was confirmed 2 days later by an elevated plasma glucose (greater than 350 mg/dl) in the fed state.

One week after injection of streptozotocin and confirmation of diabetes rats were randomly assigned to each of the following

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groups: (a) control rats on control diet (Purina Rat Chow), (b) control rats on 1% cholesterol diet (Purina Rat Chow base, ICN Nutritional Biochemicals, Cleveland, Ohio), (c) diabetic rat on control diet, (d) diabetic rat on 1% cholesterol diet. The rats were maintained on these diets for 3 weeks and then used to study aortic 6-keto-$PGF_{1\alpha}$ synthesis.

6-Keto-$PGF_{1\alpha}$ assays. [^{14}C]Arachidonic acid (55.8 Ci/mole) was obtained from New England Nuclear (Boston, Mass.) and cold arachidonic acid from Nu Chek Prep (Elysian, Minn.). Both were stored in absolute ethanol at −20°. The specific activity of the labeled arachidonic acid was diluted with unlabeled arachidonic acid to obtain the desired concentrations. The ethanol was evaporated under N_2 and the arachidonic acid reconstituted in 10−20 m*M* Na_2CO_3 to the desired concentration. Fresh solutions in Na_2CO_3 were prepared on each day the assays were performed. Approximately 0.25 μCi of [^{14}C]arachidonic acid was used per assay.

The thoracic aorta was quickly dissected following exsanguination, cleared of connective and fatty tissue, and cut into small rings 3−5 mm in length. Individual rings were placed in 0.475 ml of phosphate-buffered saline (NaCl, 8 g/liter; KCl, 0.2 g/liter; $CaCl_2$, 0.1 g/liter; $MgCl_2$ $2H_2O$, 0.1 g/liter; Na_2 HPO_4 $2H_2O$ 1.15 g/liter; KH_2PO_4, 0.2 g/liter; glucose, 1 g/liter, pH 7.2) and preincubated for 2 min at 37° prior to the addition of 25 μl of various concentrations of [^{14}C]arachidonic acid. After a 10-min incubation the reaction was stopped by adding 0.5 ml 2 *M* citric acid. Time course experiments indicated that the aortic production of 6-keto-$PGF_{1\alpha}$ was linear for at least 20 min for both control and diabetic aorta (Fig. 1). The prostaglandins were extracted with 10 ml chloroform/methanol (2:1) and the lower organic layer was collected following the addition of 1 ml 0.9% NaCl. The extract was evaporated to a small volume under N_2 and the various prostaglandins separated by thin-layer chromatography (250 μm silica gel G plates, Analabs, North Haven, Conn.) using a solvent system consisting of ethyl acetate/

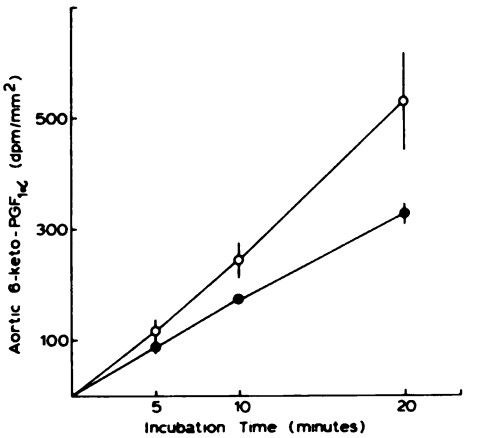

FIG. 1. Aortic 6-keto-$PGF_{1\alpha}$ synthesis (mean ± SD) as a function of incubation time for control (○, n = 4) and diabetic (●, n = 4) rats.

2,5,5-trimethylpentane/acetic acid/water (90:50:20:100, v/v/v/v, organic layer). Prior to separation, pure standards for PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ (Upjohn Co., Kalamazoo, Mich.) were added to allow visualization following a brief exposure to iodine vapor. The areas corresponding to PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ were scraped into vials, 10 ml of Aquasol 2 (New England Nuclear) added, and quantitated by liquid scintillation counting. The remaining areas were also scraped and counted to determine percentage recovery (always greater than 85%). Background counts determined from boiled aortic rings were subtracted prior to the calculation of the molar amount of product formed.

Basal aortic 6-keto-$PGF_{1\alpha}$ synthesis was measured by specific radioimmunoassay (6-keto-[^{3}H]$PGF_{1\alpha}$ kit from New England Nuclear). Aortic rings were suspended in 0.5 ml of phosphate-buffered saline and incubated at 37° for 10 min. At the end of the incubation period the solution was removed with a pipet and frozen at −20°. The concentration of 6-keto-$PGF_{1\alpha}$ was then measured using radioimmunoassay.

For both assays the amount of 6-keto-$PGF_{1\alpha}$ formed was normalized by the surface area of the aortic ring. Surface area was measured after cutting the ring open and laying it flat. Two measurements of length and width were made using a Bausch

TABLE I. BODY WEIGHT AND PLASMA GLUCOSE, TRIGLYCERIDE, AND CHOLESTEROL IN CHOW OR CHOLESTEROL-FED CONTROL AND DIABETIC RATS

Group	Body weight	Concentration (mg%)		
		Glucose	Triglyceride	Total cholesterol
Control (n = 8)	339 ± 11[a]	186 ± 10	27 ± 6	57 ± 1
Diabetic (n = 8)	229 ± 12	478 ± 17*	450 ± 91*	99 ± 3*
Control + 1% cholesterol (n = 8)	348 ± 6	177 ± 18	66 ± 11**	69 ± 3
Diabetic + 1% cholesterol (n = 4)	202 ± 18	481 ± 35*	2009 ± 388*	3175 ± 381*

[a] Results are means ± SEM.
* $P < 0.01$, as compared to the control (range test).
** $P < 0.05$.

and Lomb measuring magnifier equipped with a metric scale (one division = 0.1 mm). Surface area was calculated by taking the product of the average length and width.

Plasma lipid analysis. Plasma lipids were determined by the LRC method (14). Plasma glucose was determined by the glucose oxidase technique, using a Beckman glucose analyzer.

Statistical analysis. Statistical comparisons between means were made using the Newman–Keuls range test after analysis of variance (15). The curves drawn in Figs. 2 and 3 were fit using weighted least squares. Weights were obtained from the inverse of the variance for each arachidonic acid concentration.

Results. The mean body weights and plasma levels of glucose, triglycerides, and cholesterol for the four groups of rats are presented in Table I. Diabetes caused a significant increase in plasma triglycerides and total cholesterol. Diabetic rats fed the 1% cholesterol diet possessed greatly elevated plasma triglycerides and total cholesterol. Control rats fed the 1% cholesterol diet experienced only a small nonsignificant elevation in plasma total cholesterol.

Table II shows the aortic conversion of exogenous [^{14}C]arachidonic acid to 6-keto-PGF$_{1\alpha}$. As previously observed by other investigators, diabetes resulted in a decreased ($P < 0.05$) aortic production of 6-keto-PGF$_{1\alpha}$. Cholesterol feeding alone also resulted in a significant decrease in aortic 6-keto-PGF$_{1\alpha}$ production. The mean aortic 6-keto-PGF$_{1\alpha}$ production for cholesterol-fed diabetic rats was the lowest of all the groups, but only significantly different from the chow-fed control group.

We also studied the effect of exogenous arachidonic acid concentration on the synthesis of 6-keto-PGF$_{1\alpha}$ by aortic rings from the four groups of rats. The data are presented in Figs. 2 and 3 in the form of double reciprocal plots. The difference in the capacity of control and diabetic aorta to form 6-keto-PGF$_{1\alpha}$ was found to be dependent on the substrate concentrations. Specifically,

TABLE II. CONVERSION OF 10 μM [^{14}C]ARACHIDONIC ACID TO 6-KETO-[^{14}C]PGF$_{1\alpha}$ IN RAT AORTA

Group	Aortic 6-keto-PGF$_{1\alpha}$ synthesis (pmole/mm^2/10 min)
Control (n = 8)	2.21 ± 0.19[a]
Diabetic (n = 8)	1.61 ± 0.17*
Control + 1% cholesterol (n = 8)	1.72 ± 0.14*
Diabetic + 1% cholesterol (n = 4)	1.23 ± 0.10*

[a] Results are means ± SEM.
* $P < 0.05$, as compared to the control group (range test).

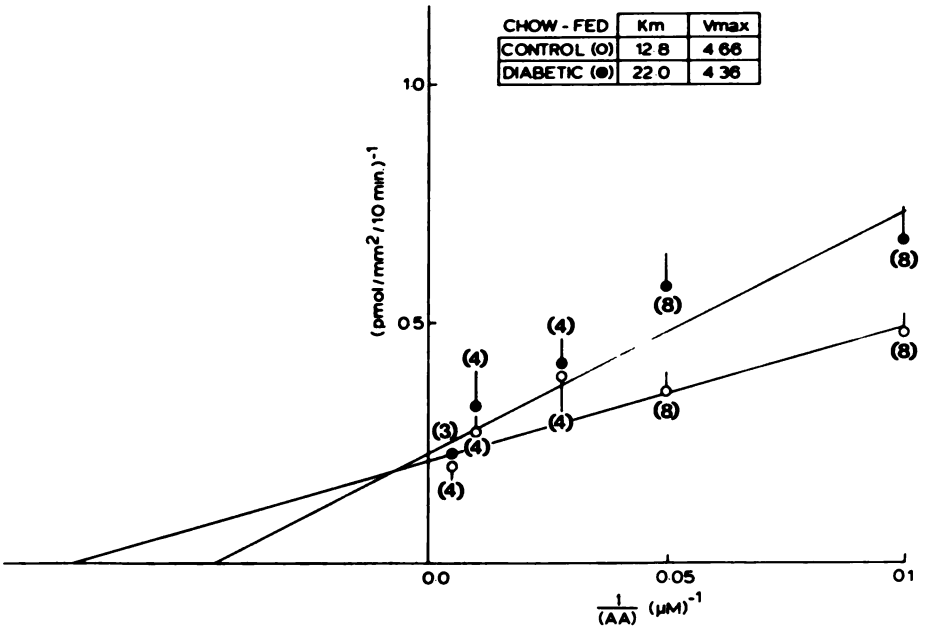

FIG. 2. Aortic 6-keto-PGF$_{1\alpha}$ synthesis (pmole/mm²/10 min) as a function of exogenous arachidonic acid concentration (AA) in control and diabetic rats. The data are presented in the form of a double reciprocal plot. The apparent maximum velocity (pmole/mm²/10 min) and K_m (μM) values were calculated from the regression lines. The number of rats are given in parentheses and the vertical bars represent the SD.

differences were more evident at lower substrate concentrations. The calculated apparent maximum velocity and apparent K_m values are given in Figs. 2 and 3. A useful comparison of the four groups can be obtained from these values. Diabetes resulted in an increased apparent K_m with no change in the apparent maximum velocity, as compared to the chow-fed control group. The apparent K_m was also increased for the cholesterol-fed control group and even more so for the cholesterol-fed diabetic group. Cholesterol-feeding also resulted in a slightly greater apparent maximum velocity for both diabetic and control rats.

Basal aortic synthesis of 6-keto-PGF$_{1\alpha}$ from endogenous arachidonic acid was also measured and found to follow a similar trend. Both cholesterol feeding and diabetes resulted in lower mean values as compared to the chow-fed group (Table III). However, the cholesterol-fed diabetic group did not possess the lowest mean value.

Discussion. The results of the present study confirm previous observations (10–13) demonstrating that the diabetic state decreases the formation of 6-keto-PGF$_{1\alpha}$ in rat aorta. The results further demonstrate that short-term cholesterol feeding (3 weeks) also results in a reduced aortic synthesis of 6-keto-PGF$_{1\alpha}$ in the adult male rat. Our studies using exogenous [^{14}C]-arachidonic acid suggested that feeding cholesterol to diabetic rats may further alter aortic 6-keto-PGF$_{1\alpha}$ synthesis. The magnitude of the change in aortic 6-keto-PGF$_{1\alpha}$ synthesis at 10 μM exogenous arachidonic acid and the apparent K_m for the cholesterol-fed diabetic group was roughly equal to the sum of the separate effects of cholesterol feeding and diabetes. Thus, the reduction in aortic 6-keto-PGF$_{1\alpha}$ synthesis found in the cholesterol-fed diabetic group may simply be an additive effect of cholesterol feeding and diabetes. An interesting finding was that diabetes appeared to result mainly in an increased apparent K_m with

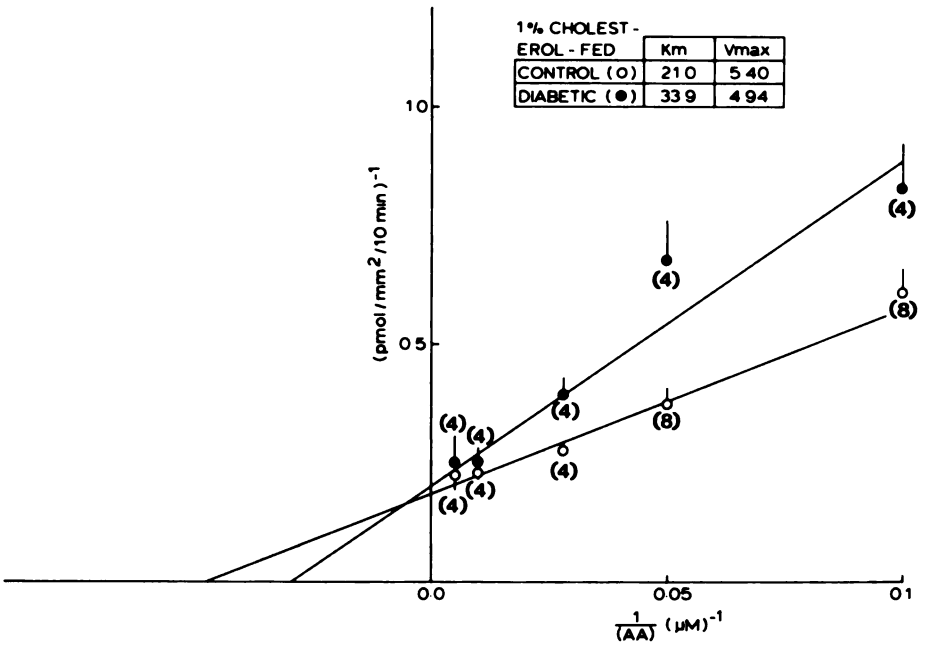

FIG. 3. Aortic 6-keto-PGF$_{1\alpha}$ synthesis (pmole/mm²/10 min) as a function of exogenous arachidonic acid concentration (AA) in control and diabetic rats fed 1% cholesterol. The data are presented in the form of a double reciprocal plot. The apparent maximum velocity (pmole/mm²/10 min) and K_m (μM) values were calculated from the regression lines. The number of rats are given in parentheses and the vertical bars represent the SD.

only minimal effect on the apparent maximum velocity. This resembles a competitive type of inhibition of aortic 6-keto-PGF$_{1\alpha}$ synthesis. Using endothelial cell cultures, Spector, *et al.* (16) found that linoleic and to some degree oleic acid inhibited the conversion of arachidonic acid to 6-keto-PGF$_{1\alpha}$ and that this inhibition appeared to be competitive in nature. Furthermore, increasing the dietary intake of linoleate by feeding corn oil to rabbits has been shown to result in a decreased aortic prostacyclin-producing capacity (17). Thus, it might be speculated that alterations in the levels of certain free or esterified fatty acids may be involved in the diabetes-induced reduction in aortic 6-keto-PGF$_{1\alpha}$ synthesis. Faas and Carter (18) found that liver microsomal Δ6 desaturase activity was depressed in diabetic rats. This depression was presumably the cause of the elevated levels of linoleate found in the liver microsomes. Serum-free fatty acids are also greatly increased in diabetic rats (19). These observations suggest that tissue linoleate would be elevated in diabetic rats.

Dietary cholesterol has also been found to alter the composition of rat liver phospholipids (20). The result was an elevation in the percentage composition of linoleate and 8,11,14-eicosatrienoate of rat liver

TABLE III. BASAL AORTIC 6-KETO-PGF$_{1\alpha}$ SYNTHESIS FROM ENDOGENOUS ARACHIDONIC ACID

Group	Aortic 6-keto-PGF$_{1\alpha}$ synthesis (pmole/mm²/10 min)
Control (n = 4)	4.07 ± 0.30[a]
Diabetic (n = 4)	2.42 ± 0.43*
Control + 1% cholesterol (n = 4)	3.31 ± 0.21
Diabetic + 1% cholesterol (n = 4)	2.58 ± 0.44*

[a] Results are means ± SEM.
* $P < 0.05$, as compared to the control group (range test).

phospholipids following the cholesterol supplementation of a control diet containing cottonseed oil. The mechanism of this alteration remains to be determined. It would be of interest to know if cholesterol feeding and diabetes alter aortic 6-keto-$PGF_{1\alpha}$ synthesis by the same mechanism. We found that the apparent K_m value for aortic synthesis of 6-keto-$PGF_{1\alpha}$ was increased for the chow-fed diabetic, cholesterol-fed control, and cholesterol-fed diabetic group. Only cholesterol feeding, however, resulted in an elevation in the apparent maximum velocity. This result suggests the possibility that cholesterol feeding may alter aortic 6-keto-$PGF_{1\alpha}$ synthesis by a mechanism different from diabetes. Further research will be necessary to resolve the mechanisms of dietary cholesterol and diabetes-induced alterations in aortic 6-keto-$PGF_{1\alpha}$ synthesis.

The results of measurements of basal aortic 6-keto-$PGF_{1\alpha}$ synthesis were similar to those using [^{14}C]arachidonic acid except that we found no additional effect of feeding cholesterol to diabetic rats. The reason for this difference is unknown. The work of Needleman *et al.* (21) suggests that the enzymes involved in the synthesis of PGI_2 and PGE_2 by the perfused kidney may exist in multiple compartments within the cell. Thus, to some extent aortic PGI_2 synthesis from endogenous arachidonic acid could involve different enzymes than synthesis from exogenous arachidonic acid. It is possible then that measurements of aortic 6-keto-$PGF_{1\alpha}$ synthesis from exogenous and endogenous arachidonic acid could yield differing results. It should be noted that aortic synthesis of 6-keto-$PGF_{1\alpha}$ from endogenous arachidonic acid (molar amount) was greater than that from 10 μM exogenous arachidonic acid (compare Tables II and III). Karpen *et al.* (22) have also published data demonstrating a greater molar production of 6-keto-$PGF_{1\alpha}$ by rat aortic rings from endogenous arachidonic acid versus 17.6 μM exogenous arachidonic acid after a 90-min incubation at 37°. In contrast the addition of exogenous arachidonic acid to bovine aortic segments and cultured endothelial cells greatly enhances PGI_2 synthesis over the basal level (23). The reason for the difference between these tissues in their 6-keto-$PGF_{1\alpha}$ synthesis from exogenous versus endogenous arachidonic acid is not known, but may be related to differences in the uptake and utilization of exogenous arachidonic acid, the basal capacity for PGI_2 synthesis, or both. Further study will be required to answer the first possibility. In regard to the latter possibility, it has been shown that PGI_2 production by rat aorta was quite high as compared to other species such as rabbit and guinea pig (24). Sinzinger *et al.* (25) have suggested that the susceptability of various species to atherosclerosis may be due to inborn differences in PGI_2 formation. The significance of the difference in our findings when using exogenous versus endogenous arachidonic acid awaits further investigation.

1. Robertson WB, Strong JP. Atherosclerosis in persons with hypertension and diabetes mellitus. Lab Invest 18:538–551, 1968.
2. Keen H. Glucose intolerance, diabetes mellitus and atherosclerosis; prospects for prevention. Postgrad Med J 52:445–451, 1976.
3. Sagel J, Colwell JA, Crook L, Laimins M. Increased platelet aggregation in early diabetes mellitus. Ann Intern Med 82:733–738, 1975.
4. Colwell JA, Halushka PV, Sarji K, Levine J, Sagel J, Nair RMG. Altered platelet function in diabetes mellitus. Diabetes 25 (Suppl 2):826–831, 1976.
5. Ziboh VA, Maruta H, Lord J, Cagle WD, Lucky W. Increased biosynthesis of thromboxane A_2 by diabetic platelets. Eur J Clin Invest 9:223–228, 1979.
6. Butkus A, Skrinska VA, Schumacher OP. Thromboxane production and platelet aggregation in diabetic subjects with clinical complications. Thromb Res 19:211–223, 1980.
7. Lagarde M, Burtin M, Berciaud P, Blanc M, Velardo B, Dechavanne M. Increase of platelet thromboxane A_2 formation and its plasmatic half-life in diabetes mellitus. Thromb Res 19:823–830, 1980.
8. Halushka PV, Rogers RC, Loadhold CB, Colwell JA. Increased platelet thromboxane synthesis in diabetes mellitus. J Lab Clin Med 97:87–96, 1981.
9. Johnson M, Harrison HE, Raferty AT, Elder JB. Vascular prostacyclin may be reduced in diabetes in man. Lancet 1:325–326, 1979.
10. Harrison HE, Reece AH, Johnson M. Decreased

vascular prostacyclin in experimental diabetes. Life Sci 23:351–356, 1978.

11. Gerrard JM, Stuart MJ, Rao GHR, Steffes MW, Mauer SM, Brown DM, White JG. Alteration in the balance of prostaglandin and thromboxane synthesis in diabetic rats. J Lab Clin Med 95:950–958, 1980.
12. Subbiah MTR, Deitemeyer D. Altered synthesis of prostaglandins in platelet and aorta from spontaneously diabetic Wistar rats. Biochem Med 23:231–235, 1980.
13. Rogers SP, Larkins RG. Production of 6-oxo-prostaglandin $F_{1\alpha}$ by rat aorta. Influence of diabetes, insulin treatment, and caloric deprivation. Diabetes 30:935–939, 1981.
14. LRC Manual of Labs Operation, Vol 1, Lipid and Lipoprotein Analysis. NHLI-DHEW publication No. 75-028, U.S. Govt Printing Office, Washington DC, 1979.
15. Snedecor GW, Cochran WG. Statistical Methods, 6th ed. Iowa State Univ Press, Ames, p271, 1967.
16. Spector AA, Hoak JC, Fry GL, Denning GM, Stoll LL, Smith JB. Effect of fatty acid modification on prostacyclin production by cultured human endothelial cells. J Clin Invest 65:1003–1012, 1980.
17. Galli C, Agradi E, Petroni A, Tremoli E. Differential effects of dietary fatty acids on the accumulation of arachidonic acid and its metabolic conversion through the cyclooxygenase and lipooxygenase in platelets and vascular tissue. Lipids 16:165–172, 1981.
18. Faas FH, Carter WH. Altered fatty acid desaturation and microsomal fatty acid composition in the streptozotocin diabetic rat. Lipids 15:953–961, 1980.
19. Paulson DJ, Crass MF. Myocardial triacylglycerol fatty acid composition in diabetes mellitus. Life Sci 27:2237–2243, 1980.
20. Morin RJ, Bernick S, Mead JF, Alfin-Slater RB. The influence of exogenous cholesterol on hepatic lipid composition of the rat. J Lipid Res 3:432–438, 1962.
21. Needleman P, Wyche A, Bronson SD, Holmberg S, Morrison AR. Specific regulation of peptide-induced renal prostaglandin synthesis. J Biol Chem 254:9772–9777, 1979.
22. Karpen CW, Pritchard KA, Merola AJ, Panganamala RV. Alterations of the prostacyclin-thromboxane ratio in streptozotocin induced diabetic rats. Prostaglandins Leukotrienes Med 8:93–103, 1982.
23. Goldsmith JC, Jafvert CT, Lollar P, Owen WG, Hoak JC. Prostacyclin release from cultured and in vivo bovine vascular endothelium. Studies with thrombin, arachidonic acid, and ionophore A23187. Lab Invest 45:191–197, 1981.
24. Tschopp TB, Baumgartner HR. Platelet adhesion and mural platelet thrombus formation on aortic subendothelium of rats, rabbits, and guinea pigs correlate negatively with the vascular PGI_2 production. J Lab Clin Med 98:402–411, 1981.
25. Sinzinger H, Clopath P, Silberbauer K, Winter M. Is the variation in the susceptability of various species to atherosclerosis due to inborn differences in prostacyclin (PGI_2) formation? Experientia 36:321–323, 1980.

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Effect of Hypertonic Sodium Chloride on Polyribosomes and Protein Synthesis of Kidneys of Rats[1] (41508)

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Abstract. The effects of a single intraperitoneal administration of hypertonic NaCl (5.3 ml of 7.55% NaCl/100 g body wt) on polyribosomes and *in vitro* protein synthesis of kidneys of rats were investigated. The results revealed that there was marked disaggregation of kidney polyribosomes of rat (fasted or nonfasted) that received a single dose of hypertonic NaCl solution 30 min prior to sacrifice in comparison to control rats that received isotonic NaCl solution. *In vitro* [^{14}C]leucine incorporation into protein was significantly decreased using either postmitochondrial supernatant or microsomes from kidneys of hypertonic NaCl-treated rats compared with control rats. Administration of a single dose of hypertonic NaCl to rats produced a significant increase in the activity of renal RNase in comparison to that of the control group. Assay of renal acid phosphatase in the particulate and soluble fractions revealed no changes in the activities in the control and experimental rats.

Earlier studies from our laboratory have reported that the administration orally or intraperitoneally of hypertonic solutions of sodium chloride, potassium chloride, magnesium chloride, sodium sulfate, or sucrose to mice rapidly induced disaggregation of polyribosomes and decreased *in vitro* and *in vivo* incorporation of [^{14}C]leucine into proteins of livers (1). In further studies, it has been demonstrated that ribosomes isolated from the livers of mice treated with a hypertonic (4%) NaCl solution were less active in initiation of polypeptide synthesis than those isolated from control animals receiving a 0.85% NaCl solution (2). Initiation factors isolated from the livers of control and experimental animals were equally active, suggesting that the ribosomes alone were affected by the hypertonicity (2). The deleterious effects of hypertonic solutions on hepatic polyribosomes and protein synthesis were shown to be mediated by a rise in the osmotic pressure of portal venous blood plasma (3).

The present investigation was designed to determine whether polyribosomes in organs other than liver will respond to changes in osmotic pressure of blood induced by administering hypertonic solutions. In this study, the effects of hypertonic NaCl injected intraperitoneally on polyribosomes and *in vitro* protein synthesis of kidneys of rats were investigated. The kidney was selected since it plays a vital role in the purification of blood and excretes excess salts derived from the blood. Furthermore, since rat serum has been reported normally to contain high levels of ribonuclease activity (4), it was decided to determine the ribonuclease activities of serum and kidney after administering hypertonic NaCl in consideration that elevations may occur which could influence the status of kidney polyribosomes and the rate of protein synthesis. The results of this communication indicate that the administration of a single dose of hypertonic NaCl to rats caused disaggregation of polyribosomes and inhibition of *in vitro* protein synthesis of kidneys along with an increased activity of renal ribonuclease.

Materials and Methods. *Animals.* Female rats of the Sprague–Dawley strain (Sprague–Dawley, Madison, Wisconsin), 11 weeks old, and weighing 170–220 g, were

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used. The rats were maintained in a temperature-controlled room with alternating 12-hr cycles of light and dark. In studies with fasted rats, the animals were fasted overnight but had free access to water. The following morning, fasted and nonfasted rats were divided into groups, each of which contained two to four animals. Rats received intraperitoneally either 0.85 or 7.55% NaCl solution (45 or 400 mg/100 g of body wt) 30 min before killing.

Chemicals. L-[^{14}C]Leucine (10 mCi/mmole) and poly-[5-^{3}H]uridylic acid (17–61 Ci/mmole) were obtained from Amersham Corporation, Arlington Heights, Illinois. Ribonuclease A, purified from bovine pancreas, was purchased from Worthington Life Sciences Division, Freehold, New Jersey.

Sucrose density gradient analysis of polyribosomes. Rats were killed by decapitation and bleeding. Kidneys were removed, dissected free of perirenal fat, and washed three times in ice-cold 0.25 *M* sucrose solution containing TKM buffer (0.05 *M* Tris–HCl, pH 7.5; 0.025 *M* KCl; and 0.01 *M* $MgCl_2$). The kidneys were weighed, minced with scissors in 4 vol of ice–cold 0.25 *M* sucrose in TKM buffer, and homogenized with a Potter–Elvehjem homogenizer with a motor-driven Teflon pestle (clearance, 0.010 in.) and four up-and-down strokes at 1325 rpm. Post-mitochondrial supernatant (PMS) was prepared by centrifuging the homogenate using a SS34 rotor in a Sorvall Model SS-3 centrifuge for 10 min at 8000 rpm. A portion (0.4 ml containing 26 $A_{260\ nm}$ units) of the PMS was layered on top of a linear 12-ml sucrose gradient (0.3 to 1.1 *M* sucrose containing TKM) in a cellulose nitrate tube, centrifuged using a Spinco SW 41 rotor in a Spinco Model L3-40 ultracentrifuge at 38,000 rpm for 1 hr at 4° and the polyribosomes were analyzed using a Gilford Model 2400S UV spectrophotometer as described earlier (3). The degree of polyribosomal aggregation of kidneys in the control and experimental groups was evaluated from the sucrose density gradients by calculating the relative distribution of monomer-dimers in relation to total ribosomes.

In vitro [^{14}C]leucine incorporation into proteins. In vitro incorporation of [^{14}C]-leucine into proteins using PMS or microsomes of kidneys was performed by the method of Nicholls *et al.* (5). Microsomes were prepared by centrifuging PMS for 1 hr at 40,000 rpm in a Spinco Model L3-40 ultracentrifuge. Microsomal pellets were resuspended in 0.25 *M* sucrose containing TKM buffer.

PMS (0.5 mg protein) or microsomes (0.25 mg protein) were incubated with 0.5 ml of standard incubation mixture in a total volume of 1.0 ml for 1 hr at 37°. The incubation mixture for each test tube contained ATP (2 μmole), phosphoenol pyruvate (10 μmole), pyruvate kinase (40 μg protein), sucrose (125 μmole), Tris–HCl, pH 7.5 (25 μmole), K^+ (12.5 μmole), Mg^{2+} (5 μmole) and [^{14}C]leucine (0.5 μCi). Cell sap (5 mg protein) from livers of control rats was used in the reaction mixture when microsomes were incubated. The cell sap was prepared by homogenizing 1 part liver and 2.5 parts 0.25 *M* sucrose containing TKM buffer and then centrifuging at 8000 rpm for 10 min. The supernatant was then centrifuged at 40,000 rpm for 2 hr. The resultant supernatant (cell sap) was used for *in vitro* incorporation of [^{14}C]leucine into protein using kidney microsomes. At the end of the incubation period, 1 ml of 10% trichloroacetic acid containing 20 mg of celite was added to each tube and processed for the measurement of radioactivity into proteins as described earlier (3).

Determination of RNase activity. The RNase activity in PMS of kidneys of control and experimental rats was determined by the method of Liu and Matrisan (4). The 200-μl final volume of reaction mixture contained 0.1 *M* Tris–HCl, pH 7.4, 5 m*M* $CaCl_2$, 0.3 m*M* dithiothreitol, 0.1 μCi [^{3}H]poly(U) containing 180,000 cpm and 10–50 μl of PMS containing 0.5 to 2.0 μg protein. At the end of 15 min incubation at 37°, the reaction was stopped by the addition of 0.6 ml of cold 95% ethanol containing 10 m*M* Mg-acetate and 0.06 mg yeast RNA in 0.58 *M* Na-acetate. The samples were kept at −20° for 1–2 hr and then centrifuged at 10,000 rpm for 15 min. Aliquots

of the supernatants were counted for the radioactivity in the enzyme hydrolyzed, ethanol-soluble nucleotides. The RNase activity (pH 7.4, 7.6, and 9.5) in the serums of control and experimental animals was determined according to the method of Rahman (6).

Determination of acid phosphatase activity. The acid phosphatase activity in the particulate and soluble fractions of kidneys of control and experimental rats was determined by the method of Tani and Ogata (7). The 3.0-ml total volume of reaction mixture contained 200 μmole of 0.2 *M* Tris–maleate buffer, pH 6.0, 10 μmole of 0.02 *M* pyridoxine, 50 μmole of 0.1 *M* *p*-nitrophenylphosphate, and 1.2–2.4 mg protein of the soluble or particulate fraction. The reaction mixture was incubated for 30 min at 37°. The reaction was stopped by addition of 3 ml of 50% trichloroacetic acid. Aliquots of 0.1 ml were diluted to 2.0 ml with distilled water, then added 2.0 ml of saturated sodium carbonate and *p*-nitrophenol formed was determined by reading the samples at optical density of 430 nm.

Chemical determinations. Protein was determined by the method of Lowry *et al.* (8) and RNA was assayed by the method of Fleck and Munro (9).

Results. In earlier studies, isolation of polyribosomes from rat kidney, using procedures that were successful with liver, yielded only small polyribosomal aggregates (5, 10). The difficulty in obtaining intact polyribosomes from kidney has been attributed to a higher level of RNase activity in kidney than in other tissues, such as liver, brain, spleen, etc. However, Zomzely *et al.* (11) have reported difficulty in the preparation of intact polyribosomes from the brain tissue, similar to that with kidney, even though brain contains relatively low levels of RNase activity (lower than in liver). These authors overcame this difficulty during the isolation of brain polyribosomes by employing high concentrations of Mg^{2+} (10–12 m*M*) (higher than used in the preparation of liver polyribosomes) and by the omission of detergent treatment. These concentrations of Mg^{2+} maintained the integrity of the polyribosomes. Therefore, in the present study, a 10 m*M* Mg^{2+} concentration was used during the preparation and sucrose gradient analysis of polyribosomes from kidney postmitochondrial supernatants. Furthermore, treatment of kidney postmitochondrial supernatant with deoxycholate, prior to layering on sucrose gradients to examine polyribosomes as in the case with the liver, was omitted. This omission of detergent treatment of renal postmitochondrial supernatant should not influence the yield of polyribosomes since, unlike in the liver, the great majority of polyribosomes in kidney of adult rats are free polyribosomes (12).

Since rat kidney has been shown to contain a high level of RNase activity, we, in the initial experiments, examined the influence of adding one of two RNase inhibitors (liver postmicrosomal supernatant and polyvinyl sulfate) during the isolation and subsequent analysis on the status of kidney polyribosomes. This was conducted to determine whether inclusion of either of these two RNase inhibitors would result in obtaining better polyribosomal profile, preparations with heavier aggregates. The profiles of kidney polyribosomes prepared in the presence of either one of the two RNase inhibitors were similar to those prepared in their absence (unpublished data). Therefore, in subsequent studies these RNase inhibitors were not included during the preparation of kidney polyribosomes. Figure 1 illustrates the profiles of polyribosomes prepared from kidneys of control rats under our experimental conditions and reveals the large polyribosomal aggregates with very few monomers and dimers. The polyribosomal patterns of the control rats appear to be similar to those reported by others (4, 10, 12).

The effects of administering a hypertonic NaCl (7.55%) solution intraperitoneally on kidney polyribosomes of fasted or nonfasted rats are presented in Fig. 1. Sucrose density gradient patterns of kidney polyribosomes 30 min after the administration of hypertonic NaCl revealed marked disaggregation of polyribosomes (an increase in dimers and monomers in relation to total ribosomes) in comparison to those

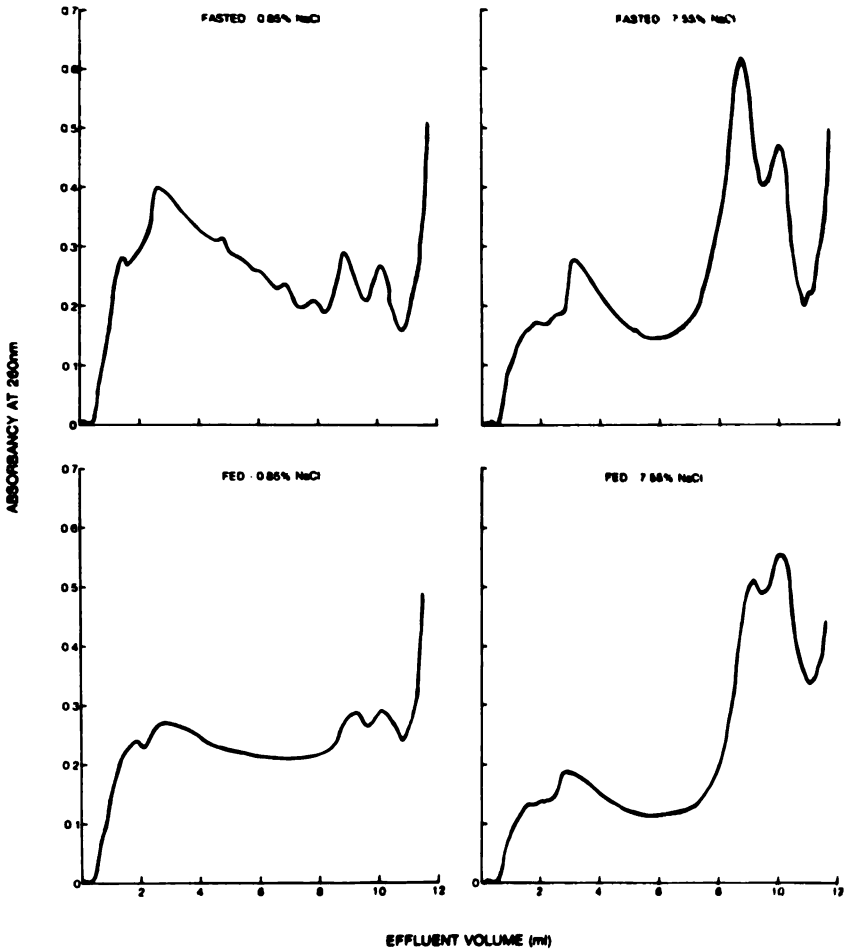

FIG. 1. Sucrose density gradient patterns of postmitochondrial supernatants of kidney polyribosomes of rats that received 0.85 or 7.55% NaCl intraperitoneally 30 min before being killed.

of control rats that received isotonic NaCl (0.85% solution). Hypertonic NaCl caused marked disaggregation of polyribosomes of kidneys of both fasted and nonfasted rats. Measurements of the areas under the polyribosomes and under the monomers plus dimers of the sucrose density gradient patterns were made in several experiments and the results are summarized in Table I. The data indicate that the administration of hypertonic NaCl to fasted and nonfasted animals resulted in a significant increase in monomer plus dimers along with a concomitant decrease in heavier polyribosomes.

In vitro [^{14}C]leucine incorporation into protein using postmitochondrial supernatants or microsomes of kidneys of control and experimental rats was also studied and the results are summarized in Table I. Both the postmitochondrial supernatants and the microsomes from kidneys of hypertonic NaCl-treated rats exhibited significant decreases in *in vitro* [^{14}C]leucine incorporation into proteins compared to values derived using preparations from control rats. Generally, the disaggregation of the renal polyribosomes correlated with the decrease in *in vitro* protein synthesizing ability in both fasted and nonfasted rats receiving hypertonic NaCl.

The administration of hypertonic NaCl

TABLE I. EFFECT OF ADMINISTERING HYPERTONIC NaCl ON WEIGHT, POLYRIBOSOMES, AND *IN VITRO* PROTEIN SYNTHESIS OF KIDNEYS

Group[a]	Weight (mg/100 g body weight)	Status of polyribosomes (Monomer − dimers/total ribosomes × 100)	[^{14}C]Leucine incorporation into protein using: Postmitochondrial supernatant (cpm/mg RNA)	Microsomes (cpm/mg RNA)
Fasted rats				
0.85% NaCl	871 ± 23 (17)[b]	31.26 ± 4.63 (5)	1479 ± 165 (11)	1878 ± 24.4 (8)
7.55% NaCl	763 ± 19* (14)	46.80 ± 2.75* (5)	457 ± 6.7* (11)	685 ± 75* (8)
% change	−12.2 ± 1.13[c,*]	+60.6 ± 20.5	−65.2 ± 5.7*	−58.1 ± 7.0*
Nonfasted rats				
0.85% NaCl	800 ± 21 (14)	32.83 ± 2.47 (6)	1614 ± 318 (6)	3971 ± 1005 (3)
7.55% NaCl	818 ± 27 (14)	52.50 ± 0.66* (6)	439 ± 122 (6)	2017 ± 420 (3)
% change	+1.72 ± 3.17[c]	+63.9 ± 11.1*	−71.5 ± 6.7*	−45.5 ± 9.1*

[a] Rats were fasted overnight or were nonfasted and then received 0.85 or 7.55% NaCl solutions (5.3 ml/100 g body weight) intraperitoneally 30 min before killing. In each experiment, kidneys of three or four rats of each group were pooled. Weights are means of the sum of both kidneys.
[b] Number of experiments in parentheses. Mean ± SEM.
[c] Mean ± SEM of differences for each experiment.
* $P < 0.05$.

did not alter the body weights of both the fasted and nonfasted rats. The mean body weights of the fasted rats were 156 ± 4.8 g (SEM) for the isotonic NaCl group and 156 ± 4.7 g for the hypertonic NaCl group, and of the nonfasted animals were 189 ± 4.7 g for the control group and 190 ± 4.6 g for the experimental group. However, the administration of hypertonic NaCl 30 min before killing caused a small, statistically significant decrease (−12%) in kidney weights in the fasted rats (Table I). On the other hand, nonfasted rats receiving hypertonic NaCl had a small insignificant increase (+2%) in kidney weights (Table I).

RNase activities of kidneys of control and hypertonic NaCl-treated rats that were fasted overnight were measured in six experiments. The results are summarized in Table II and reveal that the levels of RNase activity of the postmitochondrial supernatants were significantly higher in the kidneys of hypertonic NaCl-treated animals than the levels in the kidneys of the 0.85% NaCl-treated rats.

It has been reported earlier that rat serum normally contains a relatively high level (higher than in serum of mice) of RNase activity (4). Therefore, it was of special interest to determine whether the elevated levels of renal RNase activity in the experimental rats may be attributed to changes in the circulating serum RNase in rats treated with hypertonic NaCl. RNase activities at pH 5.4, 7.6, and 9.5 were measured in the sera of control and hypertonic NaCl-treated rats. The results of these experiments are summarized in Table III and reveal no appreciable changes in the activities of RNase in sera of the control and experimental groups.

Since the serum RNase activities were essentially unaltered after the administra-

TABLE II. RNase ACTIVITIES OF THE POSTMITOCHONDRIAL SUPERNATANTS OF KIDNEYS OF RATS THAT RECEIVED ISOTONIC OR HYPERTONIC NaCl

Group[a]	Renal RNase activities: Units/mg protein	Percentage change
0.85% NaCl	14.6 ± 3.4 (6)[b]	
7.55% NaCl	34.0 ± 7.2* (6)	+152.9 ± 42.4[c,**]

[a] Overnight fasted rats received 0.85% NaCl or 7.55% NaCl solution intraperitoneally 30 min before killing. In each experiment, kidneys of three or four rats in each group were pooled.
[b] Number of experiments in parentheses. Mean ± SEM.
[c] Mean ± SEM of differences for each experiment.
* $P < 0.05$.
** $P < 0.02$.

TABLE III. RNase ACTIVITY IN THE SERA OF RATS TREATED WITH ISOTONIC OR HYPERTONIC NaCl

Group[a]	Serum RNase activities (A_{260} units/ml)		
	pH 5.4	pH 7.6	pH 9.5
0.85% NaCl	31.97 ± 4.2 (8)[b]	36.08 ± 2.7 (13)	38.23 ± 6.4 (8)
7.55% NaCl	31.38 ± 2.2 (8)	38.35 ± 1.9 (13)	37.32 ± 6.1 (8)

[a] Overnight-fasted rats received 0.85% NaCl or 7.55% NaCl intraperitoneally 30 min before killing. Blood samples were collected by heart puncture under ether anesthesia and serum samples were separated 30 min later by centrifugation.

[b] Number of animals in parentheses. Mean ± SEM.

tion of hypertonic NaCl, we then considered the possibility that the hypertonic NaCl induced cellular injury within the kidneys by altering the osmolarity of the circulating blood. Such injury could affect the lysosomes within the renal epithelial cells and lead to an elevation in free RNase activity. To determine whether lysosomal release of enzymes may be responsible for the elevated levels of RNase activity in the kidneys of hypertonic NaCl-treated rats, we assayed for the activity of an important lysosomal enzyme, acid phosphatase, in the particulate and supernatant fractions of kidneys of control and experimental rats in three experiments. The acid phosphatase activities (A_{430} units/mg protein) of particulate and soluble fractions were, respectively, 37.0 ± 0.5 and 56.3 ± 7.7 for the isotonic NaCl group, and 44.8 ± 6.5 and 59.5 ± 12.1 for the hypertonic NaCl group. The results of these experiments indicated that the administration of hypertonic NaCl did not cause a major increase or shift in the activities of acid phosphatase in the particulate and soluble fractions compared to the corresponding fractions of the control group. This suggests that the lysosomes within the renal cells were probably unaffected by the administration of hypertonic NaCl and therefore may not be a contributory factor for the observed increase in the RNase activity of the kidneys of the experimental rats.

Discussion. The data presented in this paper demonstrate that the acute administration of a hypertonic NaCl solution caused disaggregation of polyribosomes and inhibition of protein synthesis in the kidneys. In addition, the kidneys of the experimental rats developed an increase in the activity of RNase.

Earlier studies from our laboratory have been concerned with how the liver is affected by hypertonic solutions particularly relating to the polyribosomes and protein synthesis in the livers of mice and rats. The results indicated that the oral or intraperitoneal administration of hypertonic salt solutions rapidly (within 5 min) produced an elevated osmotic pressure of the portal blood (1, 3). This increase in the portal blood osmolarity was associated with disaggregation of hepatic polyribosomes and with a decrease in both *in vitro* and *in vivo* incorporation of L-[^{14}C]leucine into hepatic and plasma proteins (1, 3). Changes in the polyribosomal profiles and in the rates of protein synthesis in response to changes in osmotic pressure caused by exposure to hypertonicity have also been observed by others in a variety of cells and organisms. Robbins *et al.* (13) and Wengler and Wengler (14) have reported that HeLa cells placed in hyperosmotic media revealed rapid disaggregation of their polyribosomes. Hsiao (15) has reported that treatment with a hyperosmotic medium caused disaggregation of polyribosomes in corn leaves. Chrispeels (16) also observed a general inhibition of protein synthesis in aleurone cells of barley by hyperosmotic media. These findings, along with our previous data with the liver and with the present findings with the kidney, suggest that protein synthesis by different cells and organs are sensitive to alterations in the osmotic pressure of the extracellular fluid.

The increased levels of RNase activity in the postmitochondrial supernatant fractions

of the kidneys of rats treated with a hypertonic NaCl solution suggests that the disaggregation of the kidney polyribosomes may be secondary due to the elevation in the levels of ribonucleases. In contrast, the polyribosomal disaggregation observed in the liver in response to the administration of 4% NaCl, reported in the earlier studies, was not found to be associated with any changes in the levels of RNase activity (2).

In an attempt to consider a mechanism by which the administered hypertonic NaCl causes polyribosomal disaggregation and decreases in *in vitro* protein synthesis in the kidney, one must consider that the elevated RNase could be of importance. Elevations of renal RNase activity in the experimental rats could be due to: (1) an increase in absorption or uptake by the kidneys of circulating RNase or (2) tissue injury causing lysosomal release of RNase. It has been reported earlier that rat serum normally contains a relatively high level (higher than in serum of mice) of RNase activity (4). Conceivably, this circulating RNase may become absorbed or extracted by the kidneys of rats treated with hypertonic NaCl. Our failure to find changes in levels of serum RNase activities in control and experimental rats in the present study does not support that the blood levels are of great importance. Yet, it is still possible that the kidneys of the experimental rats are able to extract RNase from the circulating blood without appreciably altering the levels of activities in the blood. Such a mechanism has been speculated as being responsible for the elevation of RNase in the remaining kidney after unilateral nephrectomy (17, 18) where, however, polyribosomes and protein synthesis were not depressed. Also, it is possible that the hypertonic NaCl causes increased osmolarity within the circulating blood which induces cellular injury within the kidneys. Such injury could affect the lysosomes within renal cells and cause an elevation in free RNase activity. Our failure to find an increase or shift in acid phosphatase activities of the particulate and supernatant fractions of the kidneys of control and hypertonic NaCl-treated rats does not support this possibility.

Following the administration of hypertonic NaCl, there is an elevation in NaCl concentration in the liver (1, 3) and probably also in the blood and in other organs, including the kidneys. This raises the possibility that an increase in the concentration of NaCl in the kidneys of rats treated with hypertonic NaCl may influence the assay of renal RNase activity. However, this possibility seems unlikely since the RNase activities in the sera of control and experimental rats were similar even though the NaCl concentration in the serum of the latter was elevated over that of the former group.

Further studies are needed to establish whether the disaggregation of kidney polyribosomes in hypertonic NaCl-treated rats is indeed due to elevated levels of RNase activity or to other mechanisms. Also, further studies are necessary to determine whether the disaggregation of polyribosomes and inhibition of protein synthesis of both liver and kidney by hypertonicity are mediated through similar or different mechanisms.

In the past, attempts to isolate large polyribosomes from rat kidney have been unsuccessful (5, 11). The reason for the difficulty in isolating intact polyribosomes from rat kidney compared to other tissue (e.g., rat liver or mouse kidney) is thought to be that rat kidney contains a high level of RNase activity (higher than in other tissues) (5, 19–21) and a low level of the naturally occurring RNase inhibitor (21) that stabilize polyribosomes (22). More recently Liu and Matrisian (4) reported a method to isolate RNase-free intact polyribosomes from rat kidney whereby they used an *in situ* arterial perfusion of rat kidney coupled with homogenization of the perfused rat kidney in heparin and detergents-fortified high-speed supernatant prepared from rat liver. In our hands, we were able to prepare large polyribosomes from kidneys of control rats (Fig. 1) by the employment of high concentrations of Mg^{2+} and the omission of detergent treatment during the isolation procedure. Another possible reason for obtaining heavier polyribosomes with very little degradation is that we have examined the

polyribosomal profiles using only postmitochondrial supernatants on linear sucrose gradients, thus eliminating several hours delay in the preparation of polyribosomes by the procedures normally used by others. Furthermore, our preparations of kidney postmitochondrial supernatants of control rats exhibited much lower levels of RNase activity compared to the levels in the rat kidney homogenate reported by Liu and Matrisian (4). Thus, in the present study these modifications used in our isolation procedure may have attributed to the lower levels of RNase activity in the rat kidneys and probably resulted in the recovery of heavier polyribosomes in the kidneys of control rats.

1. Lynn JK, Sarma DSR, Sidransky H. Response of hepatic polyribosomes of the mouse to the administration of anisotonic solutions. Life Sci 10:385–394, 1971.
2. Lynn JK, Murty CN, Sidransky H. Osmoregulation of ribosomal function in mouse liver. Biochim Biophys Acta 299:444–451, 1973.
3. Lynn JK, Sidransky H. Effect of changes of osmotic pressure of portal blood on hepatic protein synthesis. Lab Invest 31:332–339, 1974.
4. Liu DK, Matrisian PE. Isolation of ribonuclease-free intact polyribosomes from rat kidney. Biochim Biophys Acta 563:445–453, 1979.
5. Nicholls DM, Ryan MP, Miall SH, Cappon ID. Factors affecting protein synthesis in rat kidney ribosome preparations. Canad J Biochem 48:105–112, 1970.
6. Rahman YE. Existence of third ribonuclease in rat liver particulate. Biochim Biophys Acta 119:470–479, 1966.
7. Tani Y, Ogata K. Acid phosphatase having pyridoxine-phosphorylating activity. In: McCormick DB, Wright LD, eds. Methods in Enzymology. New York, Academic Press, Vol 18:p630, 1970.
8. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
9. Fleck A, Munro HN. The precision of ultraviolet absorption measurements in the Schmidt–Thannhauser procedure for nucleic acid estimation. Biochim Biophys Acta 55:571–583, 1962.
10. Shires TK, Ekren T, Hinderaker P, Pitot HC. Effect of ribosomal removal on rough microsomal composition and polysome attachment in vitro. Biochim Biophys Acta 374:59–75, 1974.
11. Zomzely CE, Roberts S, Gruber CP, Brown DM. Cerebral protein synthesis. II. Instability of cerebral messenger ribonucleic acid–ribosome complexes. J Biol Chem 243:5396–5409, 1968.
12. Priestly GC, Malt RA. Development of the metanephric kidney. Protein and nucleic acid synthesis. J Cell Biol 37:703–715, 1968.
13. Robbins E, Pederson T, Klein P. Comparison of mitotic phenomena and effects induced by hypertonic solutions in HeLa cells. J Cell Biol 44:400–416, 1970.
14. Wengler G, Wengler G. Medium hypertonicity and polyribosome structure in HeLa cells. The influence of hypertonicity of the growth medium on polyribosomes in HeLa cells. Eur J Biochem 27:162–173, 1972.
15. Hsiao TC. Rapid changes in level of polyribosomes in Zea-M in response to water stress. Plant Physiol 44:39–44, 1969.
16. Chrispeels MJ. Mechanism of osmotic regulation of hydrolase synthesis in aleurone cells of barley: Inhibition of protein synthesis. Biochem Biophys Res Commun 53:99–104, 1973.
17. Rabinovitz M. Plasma ribonuclease uptake by rat kidney cortex. Proc Soc Exp Biol Med 100:865–867, 1959.
18. Royce, PC. Role of renal uptake of plasma protein in compensatory renal hypertrophy. Amer J Physiol 212:924–930, 1967.
19. Roth JS. Ribonuclease. III. Ribonuclease activity in rat liver and kidney. J Biol Chem 208:181–194, 1954.
20. De Lamirande G, Allard C. Studies on the distribution of intracellular ribonucleases. Ann NY Acad Sci 81:570–584, 1959.
21. Liu DK, Matrisian PE. Regional differences in ribonuclease content of rat and mouse kidney. Biochem J 164:371–377, 1977.
22. Blobel G, Potter VR. Relation of ribonuclease and ribonuclease inhibitor to the isolation of polysomes from rat liver. Proc Nat Acad Sci USA 53:1283–1288, 1966.

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Classification of Hybridomas to Respiratory Syncytial Virus Glycoproteins (41509)

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Abstract. We have classified 28 hybridomas to the RS virus glycoproteins, VP66, VP84, by virus neutralization, RIP, and RIA tests. Without resorting to RIP, a combination of neutralization tests and RIA on BCH4 and RS/HO cells could be used to classify 79% of the hybridomas antibodies correctly. VP66 contains major determinants for virus neutralization since 11/13 hybridoma antibodies to this protein neutralized RS virus while 0/15 hybridoma antibodies to VP84 neutralized the virus.

Respiratory syncytial (RS) virus is an important respiratory pathogen of the family Paramyxoviridae (1) and is a leading cause of lower respiratory disease in young children (2). Before a successful RS virus vaccine can be developed, more information about the surface proteins is needed in order to fully define the host response to RS virus infection. Such studies have been hampered by the difficulty in obtaining purified viral proteins and monospecific antisera. We have investigated these problems by developing hybridomas to glutaraldehyde-fixed RS virus-infected HeLa cells. The initial fusion resulted in seven clones secreting monoclonal antibodies to three viral proteins (3).

The development of Balb/c cells persistently infected with RS virus (BCH4) (4) provided a convenient substrate for radioimmunoassay (RIA) and allowed us to eliminate clones secreting antibodies to human components early in the screening process. These cells potentially could induce antibodies better if injected into a syngeneic host to minimize antibody response to cellular antigen. We, therefore, injected Balb/c mice with syngeneic BCH4 cells for the production of viral antigen-specific hybridomas.

Although there has been a number of studies involved with the identification of RS virus glycoproteins (5–11) the results are not straightforward and are often contradictory. Most authors present data for three glycoproteins with molecular weights of 70,000–90,000, 40,000–50,000, and 19,000–25,000. Wunner and Pringle (11), however, could not confirm the presence of the 70,000–90,000 or 19,000–25,000 mol wt glycoproteins, but instead found two glycoproteins, mol wt 40,000–50,000 (VGP48 and VGP42). Recently the large glycoprotein was immunoprecipitated from infected cells (5, 6) but other glycoproteins, mol wt 45,000–50,000 and 17,000–24,000, were not. The development of monoclonal antibodies with specificity for the RS virus glycoproteins would greatly help in the identification and study of these glycoproteins.

Materials and Methods. *Cells and viruses.* HeLa Ohio (HO) cells were obtained from Flow Laboratories (McLean, Va.). The Balb/c cell line (BCH4), persistently infected with RS virus, has been described (4). Both cell lines were grown in equal parts of minimum essential medium with Earles salts and basal Eagle medium with Hank's salts, 10% heated (56°/45 min) fetal bovine serum (Dutchland Labs) and 4 m*M* glutamine. Suspension cultures of HO cells were grown in 10% heated fetal bovine serum, 4 m*M* glutamine in Eagles minimum essential medium, spinner modified. HO cells were infected (RS/HO) in suspension with 1 PFU/cell of RS virus, Long strain. The cells were rinsed 2 hr after infection and resuspended in suspension medium, with 5% fetal bovine serum, at 2×10^6

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cells/ml. RS/HO cells were labeled with 10 μCi/ml of D-[1,6-^{3}H]glucosamine hydrochloride (New England Nuclear, sterile aqueous solution) 12 hr postinfection. Cells and culture fluid were collected 24 hr postinfection. Virus-free culture fluid was prepared by pelleting the virus at 7500 rpm for 4 hr in a Beckman J-21C centrifuge (12).

Virus neutralization. Hybridoma culture fluids were tested for virus neutralizing antibodies as described (3) with minor modifications. Four units of guinea pig complement (Flow Laboratories) were added throughout to increase the sensitivity of the assay (13).

Indirect immunofluorescence (IFL). IFL was performed using live RS/HO cells as described (4).

Radioimmunoprecipitation (RIP). RIP using iodinated viral proteins (14), [^{3}H]-glucosamine-labeled cell lysate, or virus-free culture fluid from [^{3}H]glucosamine-labeled RS/HO cells was performed as described (14). Samples were analyzed by slab gel electrophoresis (14).

Radioimmunoassay (RIA). Indirect RIA using ^{125}I-goat anti-mouse γ globulin as the second antibody and methanol-fixed RS/HO or BCH4 cells as the substrate was recently described (3).

Somatic cell hybridizations. The generation of hybridomas followed standard techniques as described for RS virus, except viable BCH4 cells were used as the immunogen instead of glutaraldehyde-fixed RS/HO cells (3).

Results. Approximately 1000 hybridoma colonies were originally screened by RIA using methanol-fixed BCH4 cells. Ten percent appeared to be RS virus specific; these colonies were cloned and virus neutralization, RIP, IFL, and further RIA analysis using methanol-fixed BCH4 and RS/HO cells performed on the spent culture fluid.

Immunofluorescence. All of the 28 clones chosen for further study had the staining characteristics of surface proteins as determined on live RS/HO cells (4). Typical results are shown in Fig. 1.

Radioimmunoprecipitation. RIP analysis indicated that about 75% of the clones precipitated one of two viral glycoproteins (Fig. 2, Table I); designated VP84 and VP66

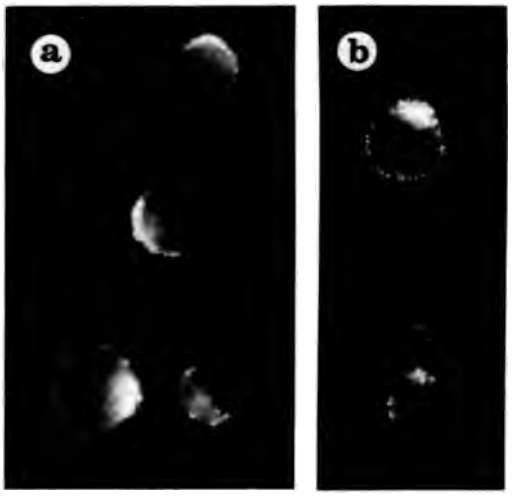

FIG. 1. Living cell immunofluorescence of RS/HO cells. RS/HO cells were prepared for living cell immunofluorescence as described (4) 22 hr after infection. The cells were incubated with (A) monoclonal 111, class 84A; (B) monoclonal 13-1 class 66A.

(14). VP84, a protein not present in great abundance on the virion (7, 14) has been identified using RIP (14). VP66 consists of two disulfide-bonded polypeptides with molecular weights of 43,000 and 19,000 (14); both polypeptides are glycosylated, VP19 relatively more so than VP43. Seventy-seven percent of the clones specific for VP66 could precipitate iodinated viral VP66 protein while only 1 of 15 (6%) clones specific for VP84 could precipitate iodinated VP84 (Table I). Seventy-three percent of the clones specific for VP84, however, precipitated [^{3}H]glucosamine-labeled VP84 (Table I). RIP using [^{3}H]-glucosamine-labeled RS/HO cell lysates showed the presence of VP66 (VP43 and VP19) when precipitated by monoclonal antibodies specific for VP66. However, VP84 and an additional glycoprotein with a mol wt of 43,000 to 46,000 precipitated from cell lysates by monoclonal antibodies specific for VP84. This protein, which is not readily detected in virus-free culture fluid, may represent a cleavage product of VP84 (Fig. 2). The width and very diffuse nature of the protein band is very similar to that obtained with VP84, but markedly different from VP43, indicating that VP43 has not coprecipitated with

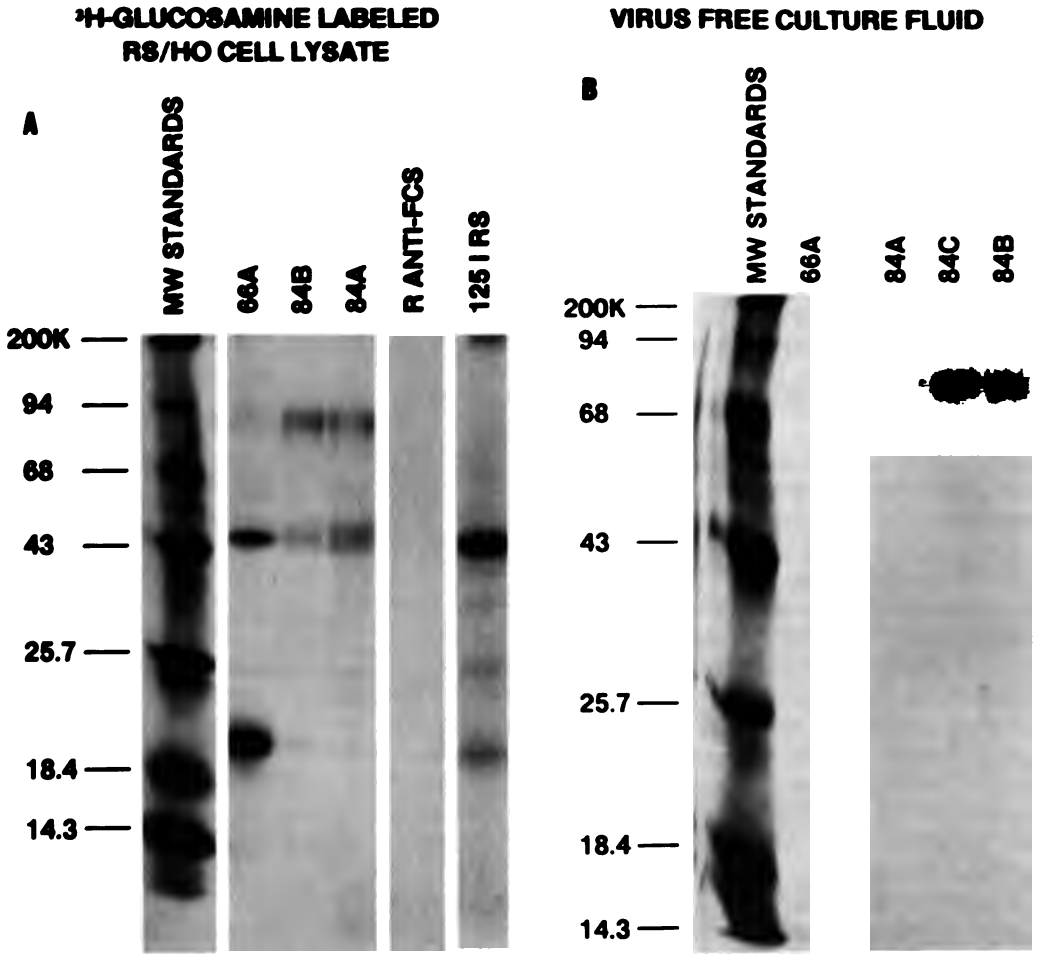

FIG. 2. RIP of RS virus-specific glycoproteins from cell lysates or culture fluid. (A) RS/HO cells grown in suspension were labeled from 12 to 24 hr postinfection with [^{3}H]glucosamine. The cells were pelleted, rinsed twice in Hank's balanced salts, 50 m*M* Hepes, pH 7.5, PMSF 100 mg/ml, and suspended in the same buffer at 5×10^7 cells/ml. Cell lysates were prepared by adding Triton X-100 to 0.1% and incubating at 37° for 30 min. Nuclei and large membrane fragments were removed and the supernatant made up to 0.1% SDS. This mixture was incubated at 45° for 30 min. Approximately 5×10^7 cell equivalents of this cell lysate were incubated with 100 μl of washed Immunoprecipitin (Bethesda Research Labs.), cleared, and incubated with the indicated antibody. The exact RIP procedure has been described (14). Samples for electrophoresis were run on slab gels consisting of 14% acrylamide with DATD as the crosslinker (14), using the discontinuous system of Laemmli (15). The specific antibodies used were as follows: class 66A, RS 13-1; class 84B, RS 18-1; class 84A, RS 111; R anti-FCS, rabbit anti-fetal calf serum (14); ^{125}I RS, iodinated RS virus proteins precipitated with rabbit anti-RS virus (14). ^{125}I-VP84 is present in immunoprecipitates with rabbit anti-RS virus (14) but is not visible in this photograph.

(B) A virus-free culture fluid preparation containing only VP84 was used to screen for monoclonals to this protein. RIP and gel electrophoresis were as described (14). The specific antibodies used were as follows: class 66A, RS 13-1; class 84A, RS 111; class 84C, RS 322; class 84B, RS 821.

VP84. Although Staphylacoccal protease treatment of culture fluid containing VP84 generates a fragment with a molecular weight of 49,000 (S. Spring, personal communication), we have not eliminated the possibility that the coprecipitating protein is due to cellular contamination. If the 40,000–45,000 mol wt protein precipitated

TABLE I. CLASSIFICATION OF HYBRIDOMAS TO RS VIRUS SURFACE PROTEINS

Class	Neut.[a]	RIP[b] V	RIP[b] S	Relative binding ratio[c]	No. of clones
66A	+	+	−	<1	6
66B	+	+	−	1–2	3
66C	+	−	−	~1	2
66D	−	+	−	<1	1
66E	−	−	−	<1	1
84A	−	+	+	2	1
84B	−	−	+	<2.5	4
84C	−	−	+	3–5	6
84D	−	−	−	2	4

[a] Viral neutralization activity scored positive if at least 50% inhibition is found at 1:20 dilution.

[b] RIP performed using: iodinated viral proteins (V), [^{3}H]glucosamine-labeled, virus-free culture fluid from RS/HO cells (S). This fluid was shown to be free of [^{3}H]glucosamine-labeled VP66 by RIP/SDS–PAGE. Viral protein precipitated is indicated by class number. See Fig. 2.

[c] Relative binding ratio is determined from comparative RIA on RS/HO and BCH4 cells per Fig. 3.

by anti-VP84 antibodies is a cleavage product, then it probably represents the VGP48 of Wunner and Pringle (11) while VP43 is their VPG42. This interpretation is based on the relative level of [^{3}H]glucosamine incorporation into VP43 and VP19 compared to the relative level of [^{3}H]glucosamine into proteins with mol wt of 40,000–50,000 and 19,000–24,000, respectively (5–8, 11).

Virus neutralization. All of the selected clones have been tested for virus neutralization activity (Table I). None of the monoclonal antibodies with neutralizing activity appears to be directed against VP84. Lack of neutralizing activity was not caused by low antibody concentration as indicated by the level of antibody binding in the RIA tests as shown below.

Radioimmunoassay. When clones were compared by RIA using BCH4 or RS/HO cells as substrate consistent patterns in the P/N binding ratios were observed (Fig. 3). These differences were used to classify clones as VP84-like or VP66-like (3). This has been useful but not entirely accurate since there is some overlap between monoclonal groups specific for the two

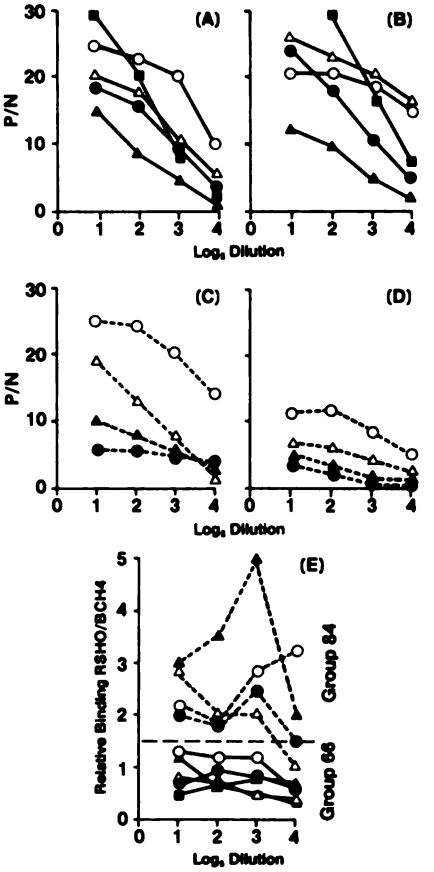

FIG. 3. Characterization of monoclonal antibodies to RS virus glycoproteins by RIA. Assay of antibodies with anti-VP66 activity (——) on RS/HO (A) and BCH4 (B) cells; monoclonal antibody and class identifications in (A) and (B) are (●) RS 284:66A, (○) RS 334:66B, (▲) RS 162:66C, (△) RS 47:66D, and (■) RS 184:66E. Assay of antibodies with anti-VP84 activity (– – –) on RS/HO (C) and BCH4 (D) cells; monoclonal antibody and class identifications in (C) and (D) are (●) RS 111:84A, (○) RS 93:84B, (▲) RS 104:84C, and (△) RS 402:84D. P/N is the cpm for the culture media samples to that for blank culture media (100–175 cpm). Antibodies assayed on uninfected HeLa or Balb/c cells exhibited P/N values ≤2. (E) The relative binding ratio was determined by dividing the P/N number obtained on RS/HO cells by the P/N number obtained on BCH4 cells.

surface proteins. In addition, monoclonals specific for RNP exhibit binding ratios similar to those for VP66 (data not shown). Further testing will be required to eliminate these problems.

Discussion. A fusion using spleenocytes from mice immunized with live BCH4 cells yielded approximately 15 stable clones secreting monoclonal antibodies to each of two surface proteins. We developed a classification scheme for these hybridomas based on IFL, virus neutralization, RIP and RIA. This classification scheme divides monoclonals to VP84 into four subgroups and those to VP66 into five subgroups. In most cases the assignment to a particular subgroup was straightforward. However, a small portion of the clones that failed to specifically precipitate a viral protein have been assigned to a subgroup based on RIA data alone and these assignments must be regarded as tentative. As knowledge about the importance of each subgroup grows, it should be possible to rapidly screen new fusions for the desired hybridomas.

In the interest of developing a rapid, simple screening technique for hybridomas to RS virus surface proteins, a variety of tests have been performed on the 28 hybridomas isolated to date. If initial screening is done using BCH4 and RS/HO cells and this is followed by virus neutralization tests, 79% of the selected clones can be placed in their correct classification. This procedure eliminates the need to screen each initial clone by the cumbersome procedures of IFL and RIP.

We are currently studying reasons for the differential binding of some classes of monoclonal antibodies to BCH4 and RS/HO cells. This may simply reflect differences in the amount of VP84/VP66 in these cells or differences in the orientation of these proteins on the cell surface.

The efficacy of using BCH4 cells as immunogen and substrate for the production and screening of hybridomas to RS virus has been established. The success obtained using BCH4 cells as an immunogen probably stems from the lack of response to BCH4 cellular antigens. In addition, the persistently infected BCH4 cells are providing a continual source of viral antigens for purification and characterization.

VP66 contains major determinant(s) involved in virus infectivity, VP84 apparently does not. We have not been able to assign a definite function to VP84, although this protein has the physicochemical characteristics of the paramyxovirus hemagglutinin (14).

The use of monoclonal antibodies with specificity for VP84 and VP66 should help clarify the complex picture of RS virus glycoproteins. The fact that two antigenically distinct proteins have very similar molecular weight raises some question as to the identity of the 45,000–50,000 mol wt glycoprotein found in nonimmunoprecipitated RS virus or infected cells (7–11). In fact, the protein that coprecipitates with VP84 may represent VGP48 as defined by Wunner and Pringle (11) while VP43 may represent their VGP42. Earlier results obtained by direct analysis of viral proteins has not yet been consolidated with more recent work involving the RIP of viral proteins (5, 6, 14). This is caused, in part, by the variable precipitation efficiency of RS virus proteins (5, 14) resulting in a selection of viral proteins that may or may not be present in great abundance. The selection process, coupled with the presence of viral proteins having similar molecular weight (e.g., VGP48, VGP42; 11), VP43, postulated cleavage product of VP84 (this paper) has produced considerable uncertainty about the number and size of the RS virus glycoproteins (5–11). This uncertainty has prevented a comprehensive understanding of RS virus glycoproteins from being developed. Hopefully, this situation will be clarified as monoclonal antibodies are used to examine the glycoproteins of RS virus more extensively.

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1. Kingsbury DW, Bratt MA, Choppin PW, Hanson RP, Hosaka Y, ter Meulen V, Norrby E, Plowright W, Rott R, Wunner WH. Paramyxoviridae. Intervirology 10:137–152, 1978.
2. Jacobs JW, Peacock DB, Corner BD, Caul EO, Clarke KR. Respiratory syncytial and other vi-

ruses associated with respiratory disease in infants. Lancet 1:871–876, 1971.
3. Cote, Jr PJ, Fernie BF, Ford EC, Shih JW-K, Gerin JL. Monoclonal antibodies to respiratory syncytial virus: Detection of virus neutralization and other antigen-antibody systems using infected human and murine cells. J Virol Methods 3:137–147, 1981.
4. Fernie BF, Ford EC, Gerin JL. The development of Balb/c cells persistently infected with respiratory syncytial virus: Presence of ribonucleoprotein on the cell surface. Proc Soc Exp Biol Med 167:83–86, 1981.
5. Bernstein JM, Hruska JF. Respiratory syncytial virus proteins: Identification by immunoprecipitation. J Virol 38:278–285, 1981.
6. Dubovi EJ. Analysis of proteins synthesized in respiratory syncytial virus-infected cells. J Virol 42:372–378, 1982.
7. Levine S. Polypeptides of respiratory syncytial virus. J Virol 21:427–431, 1977.
8. Peeples M, Levine S. Respiratory syncytial virus polypeptides: Their location in the virion. Virology 95:137–145, 1979.
9. Pringle CR, Shirodaria PV, Gimenez HB, Levine S. Antigen and polypeptide synthesis by temperature-sensitive mutants of respiratory syncytial virus. J Gen Virol 54:173–183, 1981.
10. Ueba O. Respiratory syncytial virus. II. Isolation and morphology of the glycoproteins. Acta Med Okayama 34:245–254, 1980.
11. Wunner WH, Pringle CR. Respiratory syncytial virus proteins. Virology 73:228–243, 1976.
12. Fernie BF, Gerin JL. The stabilization and purification of respiratory syncytial virus using $MgSO_4$. Virology 106:141–144, 1980.
13. Buynak EB, Weibel RE, Carlson AJ, McLean AA, Hilleman MR. Further investigations of live respiratory syncytial virus vaccine administered parenterally. Proc Soc Exp Biol Med 160:272–277, 1979.
14. Fernie BF, Gerin JL. Immunochemical identification of viral and non-viral proteins of the respiratory syncytial virus virion. Infect Immun 37:243–249, 1982.
15. Laemmli UK. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature (London) 227:680–685, 1970.

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Oligoclonal IgG in the Cerebrospinal Fluid of Guinea Pigs with Experimental Allergic Encephalomyelitis (41510)

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Abstract. Oligoclonal IgG bands were demonstrated in the cerebrospinal fluid of guinea pigs with experimental allergic encephalitis before clinical signs developed. No bands were found in the serum of these animals nor were bands found in the CSF or serum of control animals. Immunofixation techniques demonstrated that the bands were immunoglobulins of the γ G class.

Experimental allergic encephalitis (EAE) is an acute neurological disease which can be produced in guinea pigs, rats, and other animals by injection of brain tissue or extracts from brain tissue such as myelin basic protein in complete Freund's adjuvant. The disease has been proposed as a possible experimental model for multiple sclerosis (MS) (1). The presence of oligoclonal IgG bands in the cerebrospinal fluid (CSF) of patients with MS is one of the most common immunological findings in this disease (2). Most patients with optic neuritis, acute herpes encephalitis, Epstein–Barr encephalitis, subacute sclerosing panencephalitis, and neurosyphilis also have bands present (3–5). Oligoclonal IgG is present in some patients with Guillain–Barre, myasthenia gravis, and Parkinson's disease but is not present in normal individuals (3, 6, 7). We report here the occurrence of oligoclonal IgG in the spinal fluid of guinea pigs with EAE.

Materials and Methods. Guinea pig myelin basic protein (BP) was prepared as previously described (8). Adult strain 13 guinea pigs were sensitized by injection of 0.7 mg BP in complete Freund's adjuvant (3.5 mg of heat-killed mycobacteria ($H_{37}R_v$) total) or 0.1 mg BP in complete Freund's adjuvant (0.1 mg of heat killed mycobacteria) in five sites in the nuchal region and in each hind foot pad. Injection of this material causes EAE in 100% of strain 13 guinea pigs within 21 days. At the time CSF was obtained (Day 11) none of the guinea pigs had developed clinical signs of disease and no circulating antibodies to BP were detectable (9). Control animals were injected with either the same emulsion containing no BP or with an emulsion containing BP but no mycobacteria. None of the control animals displayed any clinical signs of EAE.

The guinea pigs were anesthetized using ether inhalation for cisternal puncture. The cisternal subarachnoid space was entered with a 23-gauge needle and the sample was allowed to fill the needle. CSF samples from control and EAE animals were centrifuged in a Beckman microfuge at 10,000 rpm for 2 min and carefully transferred to conical plastic vials and stored at −20°; only samples with no evidence of hemolysis or red cells were included in this study. Blood was obtained by cardiac puncture and was allowed to clot at room temperature. The sample was centrifuged and serum was stored at −20°.

A modification of the SDS–polyacrylamide gel electrophoresis was used for the determination of oligoclonal IgG (10). With this new method it is possible to test unconcentrated CSF. Briefly, the electro-

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phoresis was carried out in vertical gel slabs. Acrylamide content of the stacking gel was 5% and the separating gel was 9%. Samples (50 μl of unconcentrated CSF or serum diluted 1:200) were prepared for electrophoresis by mixing 10 parts CSF or diluted serum with one part buffer (70% sucrose, 10% SDS, and bromophenol blue as dye). These specimens were placed directly on the stacking gel with no further treatment. We tested CSF samples from EAE and control animals with comparable IgG concentrations, i.e., 3 μg of IgG contained in 50 μl CSF. Oligoclonal bands were detected in EAE animals while no bands were found in control animals. These results indicate that the appearance of oligoclonal bands is independent of the IgG concentration. Purified guinea pig IgG were used as controls. After electrophoresis with a constant current of 10 to 30 mA per gel for 3–4 hr, the slabs were removed and stained for protein with Coomassie brilliant blue. Two or more bands in the IgG region were considered positive.

Immunofixation of IgG was done on the gel surface. Strips of cellulose acetate membrane were impregnated by soaking in the appropriate undiluted antiserum (anti-guinea pig IgG heavy and light chains, H & L) prepared in goat (Cappel Laboratories). The gels were overlaid with the strips for 2 hr in a moisture chamber at 4°. Antibody excess was carefully washed out overnight in phosphate-buffered saline (PBS) (pH 7.2) at room temperature. To detect the reaction between the oligoclonal IgG and specific immune sera, the washed gel was incubated with peroxidase conjugated rabbit anti-goat IgG (Miles-Yeda LTD, Israel) for 2 hr in a moisture chamber at 4°. The gel was washed overnight with frequent changes of PBS (pH 7.4) and reacted with diaminobenzidine (11). The enzymatic reaction was stopped by washing with cold tap water when color developed in the gel. The dark brown bands demonstrated by this technique confirmed the presence of IgG bands. Side-by-side comparisons were done on identical samples in which one lane was stained with Coomassie brilliant blue and another lane with peroxidase-conjugated antisera, to prove that IgG was present.

Results. A modified SDS–polyacrylamide electrophoresis method was used to detect oligoclonal bands in the unconcentrated CSF of guinea pigs in which EAE was induced. The animals were clinically well at the time they were studied. With the sensitizations used clinical signs of EAE are observed by 21 days after inoculation. Oligoclonal IgG bands were observed in the CSF of all 14 guinea pigs obtained 11 days after inoculation with 0.7 mg BP (Table I). Three bands were detected in 5 and two bands in 9 guinea pigs using this concentration (Fig. 1). Two oligoclonal bands were also found in three of four guinea pigs which were inoculated with 0.1 mg BP. These results suggest that the variation in BP concentrations did not affect the appearance of oligoclonal bands in these animals. None of the control guinea pigs had detectable components in the IgG region of the CSF.

The electrophoretic pattern of the sera of the inoculated guinea pigs showed no difference when compared to the control groups; all of the sera were considered to have no oligoclonal IgG (Table I).

The globulin class of the oligoclonal bands was determined by immunofixation with purified anti-guinea pig IgG heavy and light chain-specific sera. The localization of the color in the approximate IgG area was observed in all samples showing specific oligoclonal IgG patterns (Fig. 1); those without oligoclonal IgG were negative. This confirmed the class of the oligoclonal IgG.

Discussion. Oligoclonal IgG bands were demonstrated in the CSF but not in the sera from 17 of 18 guinea pigs inoculated with myelin basic protein in complete Freund's adjuvant. The observation that oligoclonal IgG did not occur in the CSF of any of the control animals shows an association with EAE. Staining with anti-guinea pig IgG heavy and light chains confirmed the specificity of the bands as IgG. The appearance of oligoclonal IgG in the CSF of basic protein-sensitized guinea pigs before the onset of EAE suggests that this antibody may contribute to the pathogenesis of the disease. Similar oligoclonal bands were found in three of four guinea pigs and reported in an abstract by Rostami (12).

TABLE I. DEVELOPMENT OF OLIGOCLONAL BANDS IN CSF OF GUINEA PIGS SENSITIZED WITH BASIC PROTEIN IN COMPLETE FREUND'S ADJUVANT

	GP sensitization BP (mg)/MTbc (mg)		No. of animals	GP with oligoclonal bands	
				CSF	Sera
EAE	0.7/3.5	CFA	14	14	0
	0.1/0.1	CFA	4	3	ND
Controls	0/0	—	1	0	0
	0/3.5	CFA	6	0	0
	0.7/0	IFA	7	0	0
	0.1/0	IFA	2	0	0

Note. Abbreviations used: CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; BP, basic protein; GP, guinea pig; MTbc, mycobacteria; ND, not done.

Oligoclonal bands were also detected in brain extracts and CSF of guinea pigs with chronic relapsing experimental allergic encephalitis (R-EAE) (13). It will be important to determine the antigenic specificity of the oligoclonal IgG in the sensitized guinea pigs since this finding may clarify the possible role of the IgG in these animals and may contribute to an understanding of the importance of oligoclonal IgG in MS.

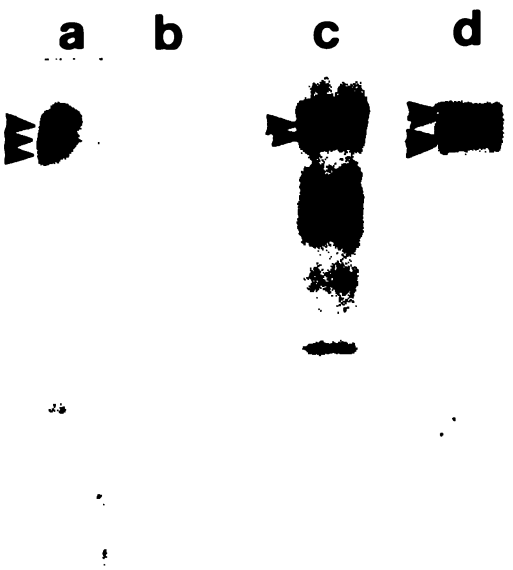

FIG. 1. Oligoclonal IgG in CSF of guinea pigs inoculated with basic protein in complete Freund's adjuvant (BP-CFA). SDS–PAGE electrophoresis of CSF. (a) CSF of guinea pig inoculated with 0.7 mg BP-CFA; (b) CSF of control guinea pig; (c) CSF of guinea pig inoculated with 0.1 mg BP-CPA; (d) immunofixation with an indirect peroxidase assay in CSF from guinea pig inoculated with BP-CFA. Note: The discrete oligoclonal bands (arrows) are in the IgG area. The IgG concentrations of CSF samples a, b, c, were similar, i.e., 3 μg IgG contained in 50 μl CSF.

A recent study of CSF and serum from rabbits with EAE showed oligoclonal IgG bands in some of these animals (14). The animals were tested prior to sensitization and at the time of development of clinical disease. A few rabbits had bands in their CSF and/or serum prior to sensitization. Of 11 rabbits given whole nervous tissue, 7 developed new bands in their serum and CSF. The absence of bands in the CSF of some of the animals in these studies and presence of bands in the serum may relate to the different species of animals studied or the collection of specimens at the time of appearance of symptoms. Our animals were sampled prior to development of symptoms and bands were always present in the CSF but not the serum. Serial studies of sensitized animals will have to be conducted to determine the time of appearance of bands. Another study (15) demonstrated anti-BP activity in the oligoclonal bands in serum and CSF from rabbits with EAE.

The use of the modified stacking polyacrylamide gel electrophoresis permitted the tests in this study to be carried out with 50-μl samples of unconcentrated CSF obtained by cisternal puncture. In the preparation of specimens for electrophoresis, the procedure avoided disruption of the IgG molecules to subunits as well as prevented IgG from aggregating during storage. This

was done by mild treatment with SDS which did not break it down to the constituent light and heavy chains. Bands are only present in the IgG region. This was further confirmed by using marker proteins of known molecular weight and by immunofixation with antibodies to guinea pig IgG heavy and light chains. The concentrations and defined porosity of the stacking and separating gels used for these studies did not permit entry of dimeric IgG (~ mol wt = 340,000 daltons) or IgM (~ mol wt = 900,000 daltons). The oligoclonal bands were the result of increased production of IgG by certain clones of plasma cells. The bands appear as distinct lines, sometimes with a background of diffuse polyclonal IgG. The SDS treatment modifies the IgG to permit it to enter the gel and migrate as a single entity. The IgG, however, is not irreversibly denatured because it can be eluted from gels without loss of functional capability. Thus, this procedure should be useful to study small serial samples from individual animals.

1. Paterson PY, Day ED, Whitacre CC. Neuro immunologic diseases: Effector cell responses and immunoregulation mechanisms. Immunol Rev 55:90, 1981.
2. Johnson KP, Arrigo SC, Nelson BJ, et al. Agarose electrophoresis of cerebrospinal fluid in multiple sclerosis: A simplified method for demonstrating cerebrospinal fluid oligoclonal immunoglobulin bands. Neurology 29:273–277, 1977.
3. Link H, Muller R. Immunoglobulins in multiple sclerosis and infections of the nervous system. Arch Neurol 25:326–344, 1971.
4. Link H, Norrby E, Olsson J. Immunoglobulins and measles antibodies in optic neuritis. N Engl J Med 289:1103–1104, 1973.
5. Olsson JE, Link H. Immunoglobulin abnormalities in multiple sclerosis. Relation to clinical parameters: Exacerbations and remissions. Arch Neurol 28:392–399, 1973.
6. Laterre EC, Callewaert A, Heremans JF, et al. Electrophoretic morphology of gamma globulins in cerebrospinal fluid of multiple sclerosis and other diseases of the nervous system. Neurology 20:982–990, 1970.
7. Williams A, Houff S, Lees A, Calne D. Oligoclonal banding in the cerebrospinal fluid of patients with postencephalitic Parkinson's. J Neurol Neurosurg Psychiatry 42:790, 1979.
8. Diebler GE, Martenson RE, Kies MW. Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. Prep Biochem 2:139–165, 1972.
9. Kies MW, Driscoll BF, Lisak RP, Alvord EC. Immunologic activity of myelin basic protein in strain 2 and strain 13 guinea pigs. J Immunol 115:75–79, 1975.
10. Iivanainen MV, Wallen W, Leon ME, et al. Micromethod for detection of oligoclonal IgG in unconcentrated CSF by polyacrylamide gel electrophoresis. Arch Neurol 38:427–430, 1981.
11. Graham RC, Karnovsky MF. The early stage of absorption of injected horseradish peroxidase in the proximal tube of the mouse kidney: Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14:291–302, 1966.
12. Rostami A, Lisak RP, Blanchard N, Guerrero F, Zweiman B, Pleasure D. Oligoclonal IgG in cerebrospinal fluid (CSF) of animals with experimental allergic encephalomyelitis (EAE). Neurology 31 (2):126–127, 1981.
13. Mehta P, Pansmann H, Wisniewski H. Immunologic studies of chronic relapsing EAE in guinea pigs: Similarities to multiple sclerosis. J Immunol 127:334, 1981.
14. Whitacre CC, Mattson DH, Paterson PY, Roos RP, Peterson DJ, Arnason BGW. Cerebrospinal fluid and serum oligoclonal IgG bands in rabbits with experimental allergic encephalomyelitis. Neurochem Res 6:87–96, 1981b.
15. Petrasek J, Panitch H, Norrby E. Formation of oligoclonal antibody in experimental allergic encephalomyelitis. Neurology 31 (2):126, 1981.

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The Effect of Dehydroepiandrosterone on Adipose Tissue Cellularity in Mice (41511)

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Abstract. The effect of dehydroepiandrosterone (DHEA) on adipose tissue growth in young adult male mice was investigated. In Experiment 1, mice were individually caged and treated by intraperitoneal injections of DHEA (25 mg/kg body weight, 3× weekly) for 20 weeks. No significant differences in body weight and adipose tissue growth were found. In Experiment 2, DHEA was administered by intubation (450 mg/kg body weight, 3× weekly). In addition, mice were housed either one/cage or five/cage. After 10 weeks of treatment body weight and epididymal and retroperitoneal fat pad weights were significantly decreased in both DHEA groups compared to their respective control groups. In both adipose depots fat cell size was significantly decreased in treated mice while there was no effect on fat cell number. No differences were found in adipose tissue lipoprotein lipase or liver fatty acid synthetase of treated mice.

The 17-ketosteroid, dehydroepiandrosterone (DHEA), has been shown by Yen *et al.* (1) and Schwartz (2) to decrease body weight gain in yellow obese mice. This alteration in body weight occurred without a concomitant change in food intake. It was also shown that DHEA treatment decreased total body lipids and the rate of liver lipogenesis (1). In addition, Yen *et al.* (1) reported that body weight was decreased in lean mice but the effect was not as dramatic as that seen in the obese mice. No additional information on lean mice was reported.

DHEA is known to inhibit the enzyme glucose-6-phosphate dehydrogenase (3–6). Inhibition of glucose-6-phosphate dehydrogenase would theoretically result in a decreased availability of NADPH for either lipogenesis or DNA synthesis. The studies in obese mice suggested that DHEA is primarily affecting lipogenesis. However, DHEA has also been shown to inhibit DNA synthesis in cultured cell lines (7) and in mouse epidermis *in vivo* (8) and to decrease the development of spontaneous breast cancer formation in C3H mice (2), indicating it can also affect cellular proliferation. It has been established that adipose tissue, the major site of stored body lipids, can change by either the size of the fat cells or the number of fat cells (9, 10). Therefore, the specific effects of chronic DHEA administration on adipose tissue growth were investigated in mice. Two modes of administration for DHEA were also studied.

Materials and Methods. *Animals.* Six-week-old male C3H mice were purchased from Jackson Laboratories, Bar Harbor, Maine. Mice were housed individually in wire mesh cages, unless otherwise indicated, and were allowed food and water *ad libitum*. A 12-h light–dark cycle was maintained and the temperature was kept at 74°F. Body weight and food intake were recorded weekly.

Experiment 1. Mice were housed individually as described and were fed Purina powdered rodent chow (5001, 5% fat by weight). One-half of the mice (n = 6/group) received intraperitoneal injections 3× weekly with 25 mg/kg body weight DHEA (Sigma Chemical Co., St. Louis, Mo.) in a homogenized suspension of saline:Emulphor (GAF, New York, N.Y.) (95:5, v/v).

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Control mice were injected with the vehicle only. After 20 weeks of treatment the mice were killed and tissue processed as described below.

Experiment 2. Mice were either housed individually (50 mice) as described or housed five per cage (30 mice) in plastic cages wtih bedding. Mice were fed Purina mouse chow (5015, 11% fat by weight). The mice individually housed received powdered food while the mice housed five per cage were given food in a pelleted form. One-half of each group were intubated with DHEA 450 mg/kg 3× weekly that was homogenized in sesame oil. Each control group received the vehicle only. Mice were treated for 10 weeks and then eight mice from each of the four groups were randomly selected and were killed and tissue processed as described below. Data presented are for the mice that were killed.

General procedures. At the end of each experimental period mice were killed by decapitation. Livers were removed for fatty acid synthetase activity determination. The livers were weighed and a (30%, w/v) homogenate was prepared in a 0.15 *M* KCl−4 m*M* $MgCl_2$, pH 7.5, solution. The homogenate was centrifuged at 10,000*g* for 15 min at 4° and the supernatant was removed and recentrifuged at 100,000*g* for 45 min at 4°. The high-speed supernatant was stored at −70°. Fatty acid synthetase activity was determined by the method of Hsu *et al.* (11). Activity was expressed as counts per minute per milligram of supernatant protein. Protein was determined by the method of Lowry *et al.* (12).

Both right and left epididymal and retroperitoneal fat pads were removed. Right and left depots were pooled from each site and weighed. Two small samples from each depot were used to determine fat cell size and number by the method of Hirsch and Gallian (13). One sample was placed in 2:1 chloroform:methanol to extract the lipids and the lipid was quantitated gravimetrically (14). The second sample was placed in a 2% osmium tetroxide mixture and incubated for 72 hr at 37°. Fixed cells were counted electronically using a Coulter Counter (Model ZB). The remainder of the adipose tissue was prepared for lipoprotein lipase activity determinations. Tissue was homogenized (20%, w/v) in 0.25 *M* sucrose−1 m*M* EDTA buffer, pH 7.4. The homogenate was centrifuged at 11,000*g* for 15 min at 4°. The postmitochondrial supernatant was removed and frozen at −70°. Lipoprotein lipase activity was determined by the method of Schotz *et al.* (15) as modified by Hietanen and Greenwood (16).

Statistical analysis. Data are presented as means ± standard deviations. Results from Experiment 1 were analyzed by Student's *t* test. Results from Experiment 2 were analyzed first by two-way analysis of variance followed by *F* test for individual comparisons (17). Level of significance was $P = 0.05$ or less.

Results. *Experiment 1.* Body weight and food intake of treated and nontreated mice are shown in Table I. As can be seen there were no differences in body weights of treated mice compared to nontreated. In addition, food intake was not affected by DHEA treatment. Liver weights and liver fatty acid synthetase activity are also shown in Table I. Neither of these factors was affected by DHEA treatment.

TABLE I. EFFECTS OF INTRAPERITONEAL DHEA TREATMENT[a] ON BODY WEIGHT, FOOD INTAKE, LIVER WEIGHT, AND LIVER FATTY ACID SYNTHETASE ACTIVITY IN MALE C3H MICE (EXPERIMENT 1)

	Body weight (g)	Cumulative food intake (g)	Liver weight (g)	FAS (cpm/mg protein)
Control	32.1 ± 2.3[b]	792.3 ± 38.3	1.7477 ± 0.2020	1.755 ± 0.493
DHEA	31.5 ± 2.6	821.0 ± 47.6	1.7298 ± 0.1977	1.570 ± 0.350

[a] Mice were treated from 6 until 26 weeks of age (25 mg/kg body weight 3× weekly).
[b] Data are means ± standard deviations; $N = 6$, both groups.

TABLE II. EPIDIDYMAL AND RETROPERITONEAL FAT PAD WEIGHTS AND FAT CELL SIZE AND NUMBER IN MALE C3H FOLLOWING INTRAPERITONEAL DHEA TREATMENT[a] (EXPERIMENT 1)

	Epididymal[b]		Retroperitoneal[b]	
	Control	DHEA	Control	DHEA
Pad weight (g)	0.9154 ± 0.2460[c]	0.7115 ± 0.3302	0.2419 ± 0.0809	0.1723 ± 0.0804
Fat cell size (μg lipid/cell	0.4915 ± 0.1852	0.5178 ± 0.2678	0.6637 ± 0.1688	0.4637 ± 0.2443
Fat cell no./ pad ($\times 10^6$)	1.3631 ± 0.4353	0.9308 ± 0.1168*	0.2670 ± 0.0696	0.2398 ± 0.0664
LPL activity[d]	3.3131 ± 3.7065	2.1784 ± 1.3883	3.2115 ± 2.3652	2.5658 ± 1.2319

[a] Mice were treated from 6 until 26 weeks of age, 25 mg/kg body weight intraperitoneally injected three times weekly.
[b] Combined right and left pads.
[c] Data are means ± standard deviations; $n = 6$, both groups.
[d] Lipoprotein lipase activity = μmole free fatty acid released $\times 10^6$ cells.
* DHEA treated significantly different from respective control groups.

Fat pad weights are shown in Table II. Both epididymal and retroperitoneal fat pad weights were reduced by DHEA treatment in comparison to nontreated control fat pad weights. However, these differences were not significant. In the epididymal fat pad, DHEA treatment did result in a significant decrease in fat cell number (Table II). No difference in epididymal fat cell size was found. In the retroperitoneal fat pad there was no change in fat cell number. There was a decrease in retroperitoneal fat cell size but this was not significant (Table II). Lipoprotein lipase acitivity in both depots was decreased by DHEA treatment (Table II). However, these differences were not significant.

Experiment 2. Body weight, weight gain, and cumulative food intake data are found in Table III. These values presented for the mice selected for further study were not different from the entire group of mice used. For example body weights of the control one/cage mice were 30.4 ± 2.9 and the body weights for the entire 25 mice in this group was 30.5 ± 2.4. DHEA administered by intubation resulted in decreased body weight in both caging situations (Table III). However, the biggest effect from DHEA treatment on body weight and weight gain was seen in DHEA five/cage mice compared to their control group. In addition, significant differences were found in the body weights of DHEA five/cage

TABLE III. BODY WEIGHT, WEIGHT GAIN, CUMULATIVE FOOD INTAKE AND COMBINED FAT PAD WEIGHTS AS A PERCENTAGE OF TOTAL BODY WEIGHT[a] IN DHEA-TREATED[b] MALE C3H (EXPERIMENT 2)

	Body weight (g)		Weight gain (g)	Cumulative food intake (g)	Combined fat pad weights, percentage total body weight
	Initial	Final			
Control 1/cage	17.8 ± 1.7[c]	30.4 ± 2.9*,†	12.6 ± 1.9*,†	327.3 ± 36.9*	3.92 ± 0.74*,†
DHEA 1/cage	16.6 ± 1.9	27.1 ± 1.5	10.9 ± 1.4	343.3 ± 24.4	2.91 ± 0.70
Control 5/cage	19.9 ± 1.6	35.1 ± 1.7*	15.6 ± 2.1*	225.3‡	4.61 ± 0.60*
DHEA 5/cage	20.1 ± 1.3	28.1 ± 2.2	8.0 ± 1.1	205.7	2.60 ± 0.54

[a] Combined epididymal and retroperitoneal fat pads (both right and left) divided by total body weight ×100.
[b] Treated with DHEA 450 mg/kg body weights from 6–16 weeks of age.
[c] All data are means ± standard deviation; $N = 8$ all groups.
* Control group significantly different from DHEA-treated group.
† Control 1/cage significantly different from DHEA 5/cage.
‡ No statistics done as no individual results were available.

mice compared to control five/cage mice from the second week of treatment while DHEA one/cage mice body weights were significantly different from control one/cage at week 1, 4, 9, and 10 (data not shown). Food intakes were not different between DHEA one/cage and control one/cage mice nor between DHEA five/cage and control five/cage mice (Table III). Both groups of five/cage mice ate less food than one/cage mice. The exact explanation for this finding is unknown. Food given to the one/cage mice was in powdered form and although great care was taken to minimize spillage by placing food in a small glass cup inside a larger cup, it is possible that over 10 weeks some unaccountable loss occurred. In addition, there may be a higher energy cost for mice housed individually as suggested by the decreased body weight in one/cage mice.

Liver weights were not different between any of the groups (Table IV). However, liver weight accounted for a significantly larger percentage of total body weight in both treated groups (Table IV). No significant differences in liver fatty acid synthetase activity were found between control and DHEA-treated groups (Table IV).

Epididymal and retroperitoneal fat pad weights of both treated groups were significantly decreased compared to their respective nontreated groups (Tables V, VI). The differences in fat pad weight were much greater than those found for body weights. For example, DHEA five/cage mice weighed 15% less than control five/cage, while epididymal fat pad weight was decreased 55%. The effect of DHEA was greater on fat pad weights in the five/cage mice than in the one/cage mice. In both treated groups the combined epididymal and retroperitoneal weights accounted for significantly less of total body weight than the combined weights of fat pads of the untreated groups (Table III).

Cellularity data for epididymal and retroperitoneal fat pads are shown in Tables V and VI, respectively. In general, the biggest effect of DHEA treatment was on fat cell size in both depots. Both DHEA one/cage and DHEA five/cage mice had significantly decreased fat cell size in epididymal and retroperitoneal fat pads compared to their respective control groups. No effect of treatment was found in epididymal fat cell number. In the retroperitoneal pad a slight but significant decrease was found in fat cell number of DHEA five/cage compared to control five/cage while no difference was found in retroperitoneal fat cell number of DHEA one/cage compared to control one/cage.

Lipoprotein lipase activity results for Experiment 2 are found in Tables V and VI. In the mice housed one/cage DHEA treatment resulted in decreased lipoprotein lipase activity in both epididymal and retroperitoneal fat pads. Slight decreases in lipoprotein lipase activity were found in both pads of DHEA five/cage mice compared to controls but these values were not significant.

Discussion. These data are in agreement with previous studies (1, 2) that have shown an obesity prevention effect of dehydro-

TABLE IV. LIVER WEIGHT, LIVER WEIGHT AS A PERCENTAGE OF BODY WEIGHT,[a] AND LIVER FATTY ACID SYNTHETASE (FAS) ACTIVITY IN DHEA-TREATED[b] MICE (EXPERIMENT 2)

	Liver weight (g)	Liver weight, percentage of body weight	FAS (cpm/mg protein)
Control 1/cage	1.6988 ± 0.2348[c]	5.5 ± 0.4*	3.463 ± 1.639
DHEA 1/cage	1.6458 ± 0.0939	5.9 ± 0.2	2.848 ± 1.111
Control 5/cage	1.6845 ± 0.1296	4.8 ± 0.2*	4.284 ± 1.528
DHEA 5/cage	1.7361 ± 0.1063	5.9 ± 0.4	5.172 ± 1.889

[a] Liver weight divided by body weight × 100.
[b] Treated with DHEA, 450 mg/kg body weight.
[c] Data all means ± standard deviations; n = 8 all groups.
* Control group significantly different from DHEA-treated group.

TABLE V. EPIDIDYMAL FAT PAD WEIGHT, CELLULARITY AND LIPOPROTEIN LIPASE ACTIVITY IN DHEA-TREATED[a] MICE (EXPERIMENT 2)

	Pad weight (g)	Fat cell size (μg lipid/cell)	Fat cell no./pad ($\times 10^6$)	LPL activity[b]
Control 1/cage[c]	0.9471*,†	0.1292*,†	4.3498	1.301*
	±0.2819	±0.0432	±0.9174	±0.315
DHEA 1/cage	0.6197	0.0711	4.2530	0.790
	±0.1817	±0.0149	±0.4931	±0.313
Control 5/cage	1.3024*	0.1827*	3.9419	2.035
	±0.2359	±0.0435	±1.2025	±0.595
DHEA 5/cage	0.5903	0.0800	4.0199	1.786
	±0.1491	±0.0213	±0.5937	±0.512

[a] Treated by intubation with 450 mg/kg body weight DHEA from 6 to 16 weeks of age.
[b] LPL activity: μmole free fatty acid released × 10^6 cells per hour.
[c] Data are means ± standard deviations; N = 8 for all groups.
* Control group significantly different from DHEA-treated group.
† Control 1/cage significantly different from DHEA 5/cage.

epiandrosterone in mice when DHEA is administered by intubation at a level of 450 mg/kg body weight. Although, Yen *et al.* (1) have reported that intraperitoneal injections of DHEA at 10 mg/kg were effective in decreasing weight gain in female yellow obese mice, in the present investigation intraperitoneal injection of DHEA at 25 mg/kg body weight did not affect body weight of lean male mice. This lack of agreement may be due to some combination of sex and strain differences as well as housing conditions, diet composition, and body weight or body fatness level. In general, DHEA seemed to have a larger effect on altering body weight when the mice were growing at a more rapid rate as shown from previous work in obese mice (1) and the present results obtained in Experiment 2 in mice housed five per cage. However, in order to more clearly differentiate the effectiveness of different modes of administration, levels of DHEA present in the treated mice should be determined and related to body weight.

DHEA appears to have no toxic effects in mice as noted by Yen *et al.* (1) and Schwartz *et al.* (18) found that mice that

TABLE VI. RETROPERITONEAL FAT PAD WEIGHT, CELLULARITY AND LIPOPROTEIN LIPASE ACTIVITY IN DHEA-TREATED[a] MICE (EXPERIMENT 2)

	Pad weight (g)	Fat cell size (μg lipid/cell)	Fat cell no./pad ($\times 10^6$)	LPL activity[b]
Control 1/cage[c]	0.2574*,†	0.1494*,†	0.9356	1.484*
	±0.0576	±0.0215	±0.1962	±0.371
DHEA 1/cage	0.1867	0.1050	0.9137	0.567
	±0.0507	±0.0360	±0.1858	±0.203
Control 5/cage	0.3198*	0.1275*	1.1225*	1.729
	±0.0650	±0.0334	±0.1524	±0.553
DHEA 5/cage	0.1817	0.0983	0.9068	1.429
	±0.0529	±0.0249	±0.1851	±0.345

[a] Treated by intubation with 450 mg/kg body weight DHEA from 6 to 16 weeks of age.
[b] LPL activity: μmole free fatty acid released × 10^6 cells per hour.
[c] Data are means ± standard deviations; N = 8 for all groups.
* Control group significantly different from DHEA-treated group.
† Control 1/cage significantly different from DHEA 5/cage.

were treated with DHEA for over one year showed no apparent side effects. In fact, DHEA treatment inhibited the development of spontaneous development of breast cancer in female C3H mice and decreased their mortality rate (18).

Although a number of metabolic inhibitors of lipogenesis have been investigated in *in vitro* or acute animal studies, few have been used in chronic studies. (−)-Hydroxycitrate, an inhibitor ATP: citrate lyase (19), is one of the exceptions. Its antilipogenic effects have been studied in both lean (20, 21) and obese rats (22, 23). However, in general its effects on weight gain and body fat seem to be mediated primarily through decreasing food intake as demonstrated from pair-feeding studies (21, 23). Therefore, DHEAs effect on decreasing weight gain without affecting food intake poses an interesting metabolic puzzle as to the disposition of the ingested calories. We are presently investigating this.

In both Experiments 1 and 2, DHEA treatment was found to decrease both epididymal and retroperitoneal fat pad weights. In Experiment 2 where the results are most consistent and significant this effect was primarily due to decreased fat cell size in both fat depots. These results are consistent with the observation that fat cell number is not usually altered in adult lean mice (24). A decrease in fat cell number was found in both the epididymal fat pad of treated DHEA mice in Experiment 1 and in the retroperitoneal fat pad of DHEA five/cage in Experiment 2. These decreases are probably due to a failure to recruit previously made fat cells which are maintained in a precursor pool until some later time (25, 26). Although recent studies have shown the ability to increase fat cell number by overfeeding (27–29), no studies have shown the ability to decrease fat cell number once they have been produced (9). In addition, it should be noted that although lean mice were used in this study, similar results would be expected in adipose depots of the yellow obese mice since this rodent's obesity is due to increased fat cell size only (24).

Lipoprotein lipase is the enzyme in adipose tissue responsible for clearing triglycerides from either chylomicra or very low density lipoproteins. A number of studies have investigated the role of lipoprotein lipase in the regulation of body weight and obesity. Elevated levels are found in obese mice (30), obese Zucker rats (31–33), and in obese humans (34). In general adipose tissue lipoprotein lipase activity correlates with fat cell size (16, 35). However, in a number of situations this relationship has been found to be uncoupled. For example, during development lipoprotein lipase activity increases prior to an increase in fat cell size (16). Steinsgrimsdottir *et al.* (36) have shown that progesterone injections also resulted in increased lipoprotein lipase activity before fat cell size was found to increase. Chronic food restriction in both obese humans (37) and obese Zucker rats (38) has been found to result in increased adipose lipoprotein lipase activity compared to normally fed controls. It has also been shown that corticosteroid treatment appears to lower lipoprotein lipase activity (39, 40). In the present experiment DHEA treatment did not lead to consistent changes in lipoprotein lipase activity. DHEA one/cage mice did have decreased lipoprotein lipase activity that paralleled the smaller fat cell size. However, in the DHEA five/cage mice there was no statistical difference in lipoprotein lipase activity between the two groups. Whether the time difference in killing these groups of mice led to diurnal variations in the enzyme activity, or hormonal factors associated with the grouped housing altered lipoprotein lipase activity independent of fat cell size changes remains to be investigated.

What role endogenous DHEA may play in the etiology or maintenance of obesity is not known. No animal studies have been done and the results of human studies have been contradictory. Lopez and Krehl (41) found that an increase in body weight in obese subjects paralleled increases in red blood cell glucose-6-phosphate dehydrogenase activity and that less DHEA was excreted in the urine. When the subjects were placed on a low carbohydrate diet a de-

creased glucose-6-phosphate dehydrogenase activity and an increase in urinary DHEA was noted. Results of another calorie-restricted study (42) showed a decrease in DHEA excretion. However it has also been reported that obese subjects have an increased clearance rate for DHEA (43). A recent study in female runners found decreased body weight to be correlated with an increase in DHEA in the blood (44). Thus, although differences in DHEA metabolism can be found in humans, clearly more research is necessary to determine the precise role of DHEA in regulation of body weight. However, clinical and epidemiological studies have shown that women who have subnormal plasma concentrations of DHEA have an increased risk of developing breast cancer (45–47). Interestingly, increased body weight is also a risk factor in breast cancer.

In conclusion the results of this study are consistent with DHEA acting as an inhibitor of glucose-6-phosphate dehydrogenase activity and of the hexose monophosphate shunt (3–6, 48, 49) resulting in a limitation of the NADPH needed for lipid synthesis. No decrease in the activity of the NADPH-dependent enzyme, fatty acid synthetase, was found *in vitro*. However, since NADPH is added to the reaction mixture, this does not exclude the possibility that fatty acid synthetase activity was reduced *in vivo* and Yen *et al.* did report a decrease in *in vivo* lipogenesis in DHEA-treated mice (1). Clearly more work is necessary to determine the effect of long-term DHEA administration on glucose-6-phosphate dehydrogenase activity and possible compensatory mechanism of providing NADPH such as malic enzyme which has been shown to increase following short-term DHEA treatment in rats (48). In addition, one would like to know where the additional calories are going which are not being laid down as lipid. Preliminary studies in rats indicate DHEA treatment may increase oxygen consumption.

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1. Yen TT, Allan JA, Pearson DV, Acton JM. Prevention of obesity in Avy/a mice by dehydroepiandrosterone. Lipids 12:409–413, 1977.
2. Schwartz AG. Inhibition of spontaneous breast cancer formation in female C3H (Avy/a) mice by long term treatment with dehydroepiandrosterone. Cancer Res 39:1129–1132, 1979.
3. Marks PH, Banks J. Inhibition of mammalian glucose-6-phosphate dehydrogenase by steroids. Proc Nat Acad Sci USA 46:447–452, 1960.
4. Kurtenbach P, Benes P, Oertel GW. On steroid conjugates in plasma. Eur J Biochem 39:541–546, 1973.
5. Oertel GW, Benes P. The effects of steroids on glucose-6-phosphate dehydrogenase. J Steroid Biochem 3:493–496, 1972.
6. Beitner R, Naor Z. The effect of adenine nucleotides and dehydroepiandrosterone on the isoenzymes of NADP+ and NAD+ glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Biochim Biophys Acta 2:437–440, 1972.
7. Henderson E, Schwartz AF, Pashko LL, Abou-Gharbia M, Swern DC. Dehydroepiandrosterone and 16-bromo-epidrosterone: Inhibitors of Epstein–Barr virus-induced transformation of human lymphocytes. Carcinogenesis 2:683–687, 1981.
8. Pashko LL, Schwartz AG, Abou-Gharbia M, Swern DC. Inhibition of DNA synthesis in mouse epidermis and breast epithelium by DHEA and related steroids. Carcinogenesis 2:717–721, 1981.
9. Hirsch J, Han PW. Cellularity of rat adipose tissue: Effects of growth, starvation and obesity. J Lipid Res 10:74–82, 1969.
10. Hirsch J, Knittle JL. Cellularity of obese and non-obese human adipose tissue. Fed Proc 29:1516–1521, 1970.
11. Hsu RY, Butterworth PHW, Porter SW. Pigeon liver fatty acid synthetase. In: Lowenstein, JM. Methods in Enzymology. New York, Academic Press, Vol 14:pp 33–34, 1969.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
13. Hirsch J, Gallian E. Methods for the determination of adipose cell size in man and animals. J Lipid Res 9:110–119, 1968.
14. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total

lipids from animal tissues. J Biol Chem 226:497–509, 1957.
15. Shotz M, Garfinkel A, Huebotter RJ, Stewart JE. A rapid assay for lipoprotein lipase activity. J Lipid Res 11:68–69, 1970.
16. Hietanen E, Greenwood MRC. A comparison of lipoprotein lipase activity and adipocyte differentiation in growing male rats. J Lipid Res 18:480–490, 1970.
17. Winer BJ. Statistical Principles in Experimental Design. New York, McGraw–Hill, 1971.
18. Schwartz AG, Hard GC, Pashko LL, Abou-Gharbia M, Swern D. Dehydroepiandrosterone: An anti-obesity and anti-carcinogenic agent. Nutrition Cancer 3:46–53, 1981.
19. Watson JA, Fang M, Lowenstein JM. Tricarballylate and hydroxycitrate: Substrate and inhibitor of ATP: Citrate oxaloacetatelyase. Arch Biochem Biophys 135:209–217, 1969.
20. Sullivan AC, Hamilton JG, Miller ON, Wheatley VR. Inhibition of lipogenesis in rat liver by (–)-hydroxycitrate. Arch Biochem Biophys 150:183–190, 1972.
21. Sullivan AC, Triscari J, Hamilton JG, Miller ON, Wheatley VR. Effect of (–)-hydroxycitrate upon the accumulation of lipid in the rat. Lipids 9:121–128, 1974.
22. Sullivan AC, Triscari J. Metabolic regulation as a control for lipid disorder. I. Influence of (–)-hydroxycitrate on experimentally induced obesity in the rodent. Amer J Clin Nutr 30:767–776, 1977.
23. Greenwood MRC, Cleary MP, Gruen R, Blase D, Stern JS, Triscari J, Sullivan AC. Effect of (–)-hydroxycitrate on development of obesity in the Zucker obese rat. Amer J Physiol 240:72–78, 1981.
24. Johnson PR, Hirsch J. Cellularity of adipose depots in six strains of genetically obese mice. J Lipid Res 13:2–11, 1972.
25. Greenwood MRC, Hirsch J. Postnatal development of adipocyte cellularity in the normal rat. J Lipid Res 15:474–483, 1974.
26. Cleary MP, Klein BE, Brasel JA, Greenwood MRC. Thymidine kinase and DNA polymerase activity during postnatal growth of the epididymal fat pad. J Nutr 109:48–54, 1979.
27. Faust IM, Johnson PR, Stern JS, Hirsch J. Diet induced adipocyte number increase in adult rats; a new model of obesity. Amer J Physiol 235:279–286, 1978.
28. Lemmonier D. Effect of age, sex and site on the cellularity of the adipose tissue in mice and rats rendered obese by a high fat diet. J Clin Invest 51:2907–2915, 1972.
29. Faust IM, Johnson PR, Hirsch J. Long term effects of early nutritional experience on the development of obesity in the rat. J Nutr 110:2027–2034, 1980.
30. Rath EA, Hems DA, Beloff-Chain A. Lipoprotein lipase activities in tissues of normal and genetically obese (ob/ob) mice. Diabetologia 10:261–265, 1974.
31. DeGasquet P, Pequignot E. Adipose tissue lipoprotein lipase activity and cellularity in the genetically obese Zucker rat (fa/fa). Biochem J 132:633–635, 1973.
32. Gruen R, Hietanen E, Greenwood MRC. Increased adipose tissue lipoprotein lipase activity during development of the genetically obese rat (fa/fa). Metabolism 27:1955–1966, 1979.
33. Boulange A, Planche E, DeGasquet P. Onset of genetic obesity in the absence of hyperphagia during the first week of life in the Zucker rat (fa/fa). J Lipid Res 20:857–865, 1979.
34. Lithell H, Boberg J. The lipoprotein lipase activity of adipose tissue from different sites in obese women and relationship to cell size. Int J Obesity 2:47–52, 1978.
35. Bjorntorp P, Enzi G, Ohlson R, Persson B, Sponbergs P, Smith U. Lipoprotein lipase activity and uptake of exogenous triglycerides in fat cells of different size. Horm Metab Res 7:230–237, 1975.
36. Steingrimsdottir L, Brasel J, Greenwood MRC. Hormonal modulation of adipose tissue lipoprotein lipase may alter food intake in rats. Amer J Physiol 239:162–167, 1980.
37. Schwartz RS, Brunzell J. Increased adipose-tissue lipoprotein-lipase activity in moderately obese men after weight reduction. Lancet 1:1230–1231, 1978.
38. Cleary MP, Vasselli JR, Greenwood MRC. Development of obesity in Zucker obese (fa/fa) rat in absence of hyperphagia. Amer J Physiol 238:284–292, 1980.
39. DeGasquet P, Pequignot E. Changes in adipose tissue and heart lipoprotein lipase activities and in serum glucose, insulin and corticosterone concentrations in rats adapted to a daily meal. Horm Metab Res 5:440–443, 1973.
40. Krotkiewski M, Bjorntorp P, Smith U. The effect of long-term dexamethasone treatment on lipoprotein lipase activity in rat fat cells. Horm Metab Res 8:245–246, 1976.
41. Lopez SA, Krehl WA. A possible interrelation between glucose-6-phosphate dehydrogenase and dehydroepiandrosterone in obesity. Lancet 2:485–487, 1967.
42. Hendrix A, Heyns W, DeMour P. Influence of a low-calorie diet and fasting on the metabolism of dehydroepiandrosterone sulfate in adult obese subjects. J Clin Endocrinol 28:1525–1533, 1968.
43. Feher T, Halmay L. The production and fate of

adrenal DHEA in normal and overweight subjects. Horm Metab Res 6:303–304, 1975.
44. Baker ER, Mathur RS, Kirk RF, Williamson HO. Female runners and secondary amenorrhea: Relation with age, parity, mileage and plasma hormonal and sex hormone binding globulin concentrations. Fertil Steril 35:247–248, 1981.
45. Bulbrook RD, Hayward JL, Spicer CC. Relation between urinary androgen and corticoid excretion and subsequent breast cancer. Lancet 2:395–398, 1971.
46. Rose DP, Stauber P, Thiel A, Crawley JJ, Milbrath JR. Plasma dehydroepiandrosterone sulfate, androstenedione, and cortisol, and free cortisol excretion in breast cancer. Eur J Cancer 13:43–47, 1977.
47. Thijssen JHH, Poortman J, Schwartz F. Androgens in postmenopausal breast cancer: Excretion, production and interaction with estrogen. J Steroid Biochem 6:729–734, 1975.
48. Tepperman HM, De La Graza SA, Tepperman J. Effect of dehydroepiandrosterone and diet protein on liver enzymes and lipogenesis. Amer J Physiol 214:1126–1132, 1968.
49. Ziboh VA, Drieze MA, Hsia SL. Inhibition of lipid synthesis and glucose-6-phosphate dehydrogenase in rat skin by dehydroepiandrosterone. J Lipid Res 11:346–354, 1970.

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Effect of Prolactin on Galactose-Induced Cataractogenesis in the Rat (41512)

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Abstract. The hypothesis was tested that the hormone prolactin, a noncataractogenic agent, can influence cataractogenesis in rats fed on a D-galactose-rich diet. This hormone accelerated the cataractogenic process in females but was without significant effect in males. It was also found that the galactose diet itself induced cataract formation more rapidly in females than in males. Thus, prolactin may play a hitherto unsuspected role in acceleration of cataractogenesis, and this role is apparently influenced by the sex of the individual.

Both in humans and in animal models, a wide variety of conditions is known to stimulate the development of cataracts in the mammalian lens. Although the initiating factors may be different, one common feature of many types of cataract is a dramatic change in lens hydration (1). A number of hormones have actions which directly or indirectly affect water balance and, as a consequence, may affect the lenticular opacification triggered by other agents. One such hormone is prolactin, which is secreted by the anterior pituitary gland and which influences water and/or electrolyte balance in a large number of vertebrates (2–4). This hormone has not been implicated in the cataractogenic process. However, we decided to test the hypothesis that prolactin, of itself a noncataractogenic agent, can influence the progression of cataractogenesis in an animal system where lens opacification is induced by a D-galactose-rich diet. The results of our investigations are reported here.

Materials and Methods. The experiment described was performed twice using identical groupings and treatments. In each run, a total of 36 Sprague–Dawley rats (18 males and 18 females), initial body weight 88–104 g, were used. The animals were separated by sex and randomly assigned to one of two groups. They were kept, three per 50 × 50 × 30-cm suspended wire cage, at 22 ± 1° under a 12-hr light/12-hr dark lighting regimen. All animals were fed *ad libitum* on a diet of 30% D-galactose and 70% ground Purina Rodent Laboratory chow without preservative. The diet was freshly prepared once a week by pulverizing the commercial food pellets and mixing it with D-galactose in a blender. The prepared diet was stored at 4°. A powdered food chow was not used in order to avoid the possible effect of preservatives (e.g., the antioxidant 2, (3)-t-butyl-4-hydroxyanisole) in the diet.

Ovine prolactin (NIH-P-S12, relative potency 35 IU/mg) was dissolved in 0.9% normal saline at a concentration of 10 IU/0.1 ml and stored at 4°. Small amounts were prepared each time so that the storage time did not exceed one week. Half of the animals (nine males and nine females) in each run received daily intraperitoneal injections of 10 IU prolactin. The other half received 0.1 ml of the saline vehicle only. The animals were weighed seven times during the experiment: on the first day, on the seventh day, and every 3 days thereafter. Food consumption was determined by weighing the food dishes every 2 or 3 days.

The eyes of all the animals were examined daily under 200 W fluorescent light. The degree of opacity was assessed subjectively and staged in accordance with the

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progression of lens opacity as described by Sippel (5).

The assessment of lens opacity was made by an experienced observer without knowledge of the experimental grouping. Two other observers also assessed the condition of the lens before a rat was returned to its cage. In cases where there was disagreement, the lower numerical stage was always used.

We expanded Sippel's scale as shown in Table I in order to minimize the chances of over- or underestimating the progress of cataractogenesis. These stages of cataractogenesis have been correlated with gradually occurring protein changes (6). For quantitation, assigned opacity scores (Table I) for both eyes of all animals were used in the statistical analyses. The experiment was terminated on the 24th day.

Results. During both runs of the experiment, cataractogenesis progressed most rapidly in prolactin-injected females. Because of the novelty of the findings from the first run of the experiment, we repeated the experiment (randomizing examination of animals and scoring lens opacity more precisely) to substantiate our results. The data from the second experimental run are presented here.

The first distinct changes in lenticular opacity were observed in two prolactin-treated females on the 12th day of treatment. By the 15th day, every animal had developed some nuclear opacity in at least one eye. At the termination of the experiment, cataracts had developed to stage 5 in only three animals, all of which were prolactin-treated females.

After 18 days of treatment, it was obvious, even to the casual observer, that cataract progression was more advanced in one of the groups than in the others (Fig. 1). This group consisted of the prolactin-treated females. Statistical analysis by group comparisons of the assigned scores corroborated the subjective assessment that there were significant differences among the four groups. These differences were apparent as early as Day 15 (analysis of variance F value = 7.28; $P = 0.0007$) and continued until the day the experiment was terminated ($F = 16.05$; $P = 0.0001$). Because the variance indicated that scores were influenced by both prolactin and sex, one-tailed t tests were performed to assess the effect of each of these parameters separately. At every time period from the 15th day onward, scores of the prolactin-treated females were found to be significantly different ($P \leq 0.045$) from the saline-injected control females. Furthermore, significant differences existed between the scores of prolactin-treated males and prolactin-treated females at every time period ($P \leq 0.011$). Only the prolactin-treated female group was consistently different from the other groups. Control male and female scores were not significantly different until Day 23. However, the mean daily scores of control males tended to be lower than those of females throughout the experiment and were significantly lower on Days 23 and 24 ($P \leq 0.03$). Prolactin appears to have little effect on the progress of cataractogenesis in males and had no effect on food consumption and body weight gain (data not presented).

Discussion. The results clearly show that prolactin accelerates cataractogenesis in female rats fed on a 30% galactose diet. Since prolactin has not been implicated

TABLE I. STAGES OF OPACITY: EQUIVALENT CATARACTOUS STAGES AS DESCRIBED BY SIPPEL (5) AND ASSIGNED OPACITY SCORES

Stages of opacity	0	1^-	1	1^+	2^-	2	2^+	3^-	3	3^+	4^-	4	4^+	5^-	5	5^+
Sippel (5) equivalent	Normal	1–A	1–B	1–C		2			3			4			5	
Assigned opacity score	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

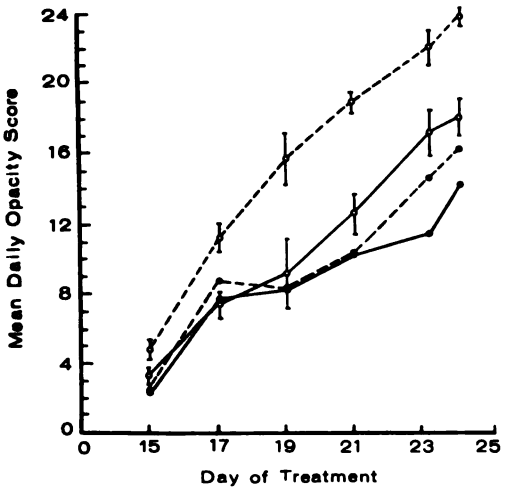

FIG. 1. Progression of lenticular opacification in 30% galactose-fed rats treated with prolactin. The mean daily opacity scores for each group (group N = 9) are shown. Mean daily opacity score represents the mean for two eyes per animal. Differences between prolactin-treated males and females, as well as differences between prolactin-treated and control females, are statistically significant (t test, $P \leq 0.045$) at all time periods. Differences between control males and females are statistically significant (t test, $P \leq 0.03$) on Days 23 and 24. (●- - -●) Prolactin-treated males; (●——●) control males; (○- - -○) prolactin-treated females; (○——○) control females. Vertical bars = standard error of mean.

previously in cataractogenesis, our findings add to the already long list of reported prolactin actions, many of which derive from the influence of prolactin on osmoregulatory physiology (2–4). Although we have not demonstrated the mechanisms by which prolactin accelerates galactose-induced cataractogenesis, we believe that this effect is also mediated by the prolactin influence on ion and water balance.

Prolactin is reported to alter water and ion movement in a number of vertebrates (2–4, 7–10) and to affect sugar and amino acid transport by the rat intestine (11). Additionally, there is evidence that prolactin may affect human glucose tolerance under certain pathologic conditions (12, 13). In galactose-induced cataractogenesis, the osmotic change that results from sugar alcohol accumulation is followed by increased lens permeability to amino acids and cations (1). Thus, although prolactin is not, by itself, a cataractogenic agent, it may accelerate the failure of the cation pump in this animal model by its action on water and cation (or even sugar and amino acid) balance.

The present studies also indicate that female rats are more susceptible to galactose-induced cataracts than are males. This cataractogenic propensity of the female was unexpected and the present experiment does not provide any basis for an explanatory hypothesis. In 1968, however, Lambert (14) reported that progestins and estrogens increase lens permeability to cations *in vitro*. The exaggerated response seen in prolactin-treated females may have been due to stimulation of prolactin secretion by estrogen (15) or it may indicate a synergistic interaction between prolactin and estrogen. Interactive effects between prolactin and other hormones are well known (4). Whatever the mechanism is that accounts for our findings, the present studies suggest that sex and prolactin are potentially important factors in cataractogenesis.

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1. Kinoshita JH. Mechanisms initiating cataract formation. Invest Ophthalmol 13:713–724, 1974.
2. Nicoll CS. Role of prolactin in water and electrolyte balance in vertebrates. In: Jaffe R, ed. Current Endocrinology. New York, Elsevier/North-Holland, pp127–166, 1981.
3. Bern HA. Prolactin and osmoregulation. Amer Zool 15:937–948, 1975.
4. Ensor DM. Comparative Endocrinology of Prolactin. London, Chapman & Hall, 1978.
5. Sippel TO. Changes in the water, protein, and glutathione contents of the lens in the course of galactose cataract development in rats. Invest Ophthalmol 5:568–575, 1966.
6. Ocken PR, Fu S-CJ, Hart R, White JH, Wagner BJ, Lewis KE. Characterization of lens protein. 1. Identification of additional soluble fractions in rat lenses. Exp Eye Res 24:355–367, 1977.
7. Mainoya JR. Effects of bovine growth hormone,

human placental lactogen and ovine prolactin on intestinal fluid and ion transport. Endocrinology 96:1165–1170, 1975.

8. Mattheij JA, Sterrenberg L, Swarts JH. Current concepts. III. Prolactin and calcium metabolism in the rat. Life Sci 27:2031–2035, 1980.
9. Bisbee CA. Prolactin effects on ion transport across cultured mouse mammary epithelium. Amer J Physiol 240:C110–C115, 1981.
10. Pang PKT. Pituitary and prolactin influences on calcium regulation in the mud puppy, *Necturus maculosus*. Gen Comp Endocrinol 44:524–529, 1981.
11. Mainoya JR. Effect of prolactin on sugar and amino acid transport by the rat jejunum. J Exp Zool 192:149–154, 1975.
12. McGarry EE, Beck JC. Biological effect of non-primate prolactin and human placental lactogen. In: Wolstenholme GEW, Knight J, eds. Lactogenic Hormones. Edinburgh, Churchill–Livingstone, p36, 1972.
13. Landgraf R, Landgraf-Leurs MM, Weissmann A, Hörl R, von Werder K, Scriba PC. Prolactin: A diabetogenic hormone. Diabetologica 13:99–104, 1977.
14. Lambert BW. The effects of progestins and estrogens on permeability of the lens. Arch Ophthalmol 80:230–234, 1968.
15. Stone JP, Holtzman S, Shellabarger CJ. Neoplastic responses and correlated plasma prolactin levels in diethylstilbestrol-treated ACI and Sprague–Dawley rats. Cancer Res 39:773–778, 1979.

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Electrophysiologic Effects of Verapamil on Cells Bordering Healed Myocardial Infarction in the Cat[1] (41513)

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Abstract. Verapamil (1 μg/ml) completely abolished a population of markedly depressed action potentials of cells (BZ-1) in border zones around healed infarcts. Even at higher concentrations, verapamil had no such inhibitory effect on other border zone cells (BZ-2), normal and central infarct zones cells. BZ-1 cells demonstrated a severely depressed $\dot{V}_{max}$ (<20 V/sec), accompanied by partial depolarization, while BZ-2 cells were characterized by abbreviated duration. Verapamil only significantly depressed $\dot{V}_{max}$ of BZ-1 cells. Action potential amplitude and action potential duration at 50% repolarization of all cells were abbreviated by verapamil. These results indicate slow inward currents may be involved in the genesis of action potentials in BZ-1 cells.

We recently reported significant action potential abnormalities in border zone ventricular muscle cells after healing of myocardial infarction in cats (1). Border zone cells are often depolarized and demonstrated a range (5.0–115.0 V/sec) in reduction of maximum rate of action potential upstroke velocity ($\dot{V}_{max}$). Slowing of $\dot{V}_{max}$ may be due to partial inactivation of a rapid Na^+-dependent current, activation of a slow inward current, or some combination of these currents (2–4). Verapamil exerts inhibitory action on the slow inward current across the myocardial membrane (5, 6). In the present study, we used verapamil to estimate the contribution of the slow channel to the generation of action potentials with depressed $\dot{V}_{max}$ in cells bordering healed myocardial infarction scars.

Materials and Methods. Acute myocardial infarction was created in 12 cats by single-stage ligation of two or three distal tributaries of the left coronary system (7). Surviving animals were maintained for 2–7 months. On the day of terminal studies, the heart was removed and dissected in cool, oxygenated, modified Tyrode's solution (composition in m*M*: NaCl = 129, $NaHCO_3$ = 20, dextrose = 5.5, KCl = 4.0, NaH_2PO_4 = 1.8, $MgCl_2$ = 0.5, $CaCl_2$ = 2.7). The atria and right ventricle were removed and the left ventricle was opened by an incision through its free wall between the two papillary muscles. The mitral valve was removed and the aortic ring opened. Preparations of left ventricle were mounted endocardial surface up in a lucite tissue bath and superfused with warmed (37°) Tyrode's solution equilibrated with 95% O_2–5% CO_2 (7).

Driving stimuli at a cycle length of 800 msec were delivered to the left bundle branch (unless noted elsewhere) through Teflon-coated silver wire electrodes, 0.01 in. in diameter. Pulse duration was 3 msec and current intensity was 1.5 times late diastolic threshold. While visible scars were almost always present over the infarcted area, surface electrograms, recorded through silver wire electrodes, were used to delineate areas of electrophysiologic abnormality (7).

Following identification of endocardial

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areas with abnormal surface electrogram, transmembrane potentials were recorded from ventricular muscle cells overlying and bordering healed myocardial infarction scars and from normal areas in the same heart, using standard microelectrode techniques (8). The glass microelectrodes filled with 3 *M* KCl (10–30 MΩ resistance) were connected through Ag–AgCl junctions to electrometers with input capacity neutralization (Bioelectric NF-1); their outputs were displayed on oscilloscope and recorded on film. Grids (6–12 mm^2) were constructed to provide a consistent sampling technique. Action potential characteristics were measured from the photographic records. $\dot{V}_{max}$ was electronically differentiated using a calibrating signal of known voltage and duration (9).

After random sampling, 3–10 representative cells from normal, infarct, and border zones in which continuous impalement could be maintained were selected for drug studies. An equilibration period of at least 60 min was allowed before initiating exposure to *l*-verapamil (Knoll). The data reported here represent the effects of drugs on maintained impalements of single ventricular muscle cells. For comparisons of the differences between mean data from central infarct, border and normal zones, an analysis of variance was employed (10). Comparisons of sample means between pretreatment and treatment of verapamil were made, employing the Student's *t* test for paired data (11).

Results. Action potentials with normal configuration were recorded from uninfarcted zones of the coronary-ligated hearts, while cells overlying the healed infarct were characterized by action potentials with prolonged duration (Table I). Border zone (~1-mm region surrounding the infarct) cells generated action potentials with varying degrees of depression of $\dot{V}_{max}$. Two distinct populations of border zone (BZ) cells became evident during exposure to verapamil. Action potentials of BZ-1 cells were completely abolished within 30 min exposure to 1 μg/ml verapamil despite stimulation at increased intensity, 0.5 mm from the recording site. These

TABLE I. COMPARISON OF THE ACTION POTENTIAL CHARACTERISTICS OF STIMULATED VENTRICULAR MUSCLE[a] FIBERS AT NORMAL, BORDER, AND INFARCT ZONES[b]

	Resting potential (−mV)	Action potential amplitude (mV)	APD_{50} (msec)	APD_{90} (msec)	$\dot{V}_{max}$ (V/sec)
NZ cell (8)[c]	82.3 ± 3.6[d]	103.4 ± 3.8	88.4 ± 7.2	128.7 ± 5.0	143.8 ± 23.8
IZ cell (8)	83.1 ± 2.4	108.1 ± 3.3	113.5 ± 6.8*	175.6 ± 10.3*	139.8 ± 15.9
BZ-1 cell (5)	51.5 ± 4.1*,†	57.5 ± 4.8*,†	60.8 ± 10.1*,†	121.1 ± 20.4†	11.4 ± 2.3*,†
BZ-2 cell (7)	66.0 ± 1.8*,†,‡	80.3 ± 4.7*,†,‡	45.2 ± 5.5*,†,‡	82.3 ± 9.6*,†,‡	82.2 ± 10.7*,†,‡

[a] Preparations were stimulated at a cycle length of 800 msec.

[b] NZ, normal zone; IZ, central infarct zone; BZ-1, border zone type 1; BZ-2, border zone type 2; APD_{50}, action potential duration at 50% repolarization; APD_{90}, action potential duration at 90% repolarization; $\dot{V}_{max}$, maximum rate of rise of phase 0 depolarization.

[c] Number of cats in parentheses; 3–10 cells per category (NZ, IZ, BZ-1, BZ-2) per cat.

[d] Data expressed as mean ± SE.

* Significant differences from normal, via analysis of variance ($P < 0.05$).

† Significant differences from central infarct, via analysis of variance ($P < 0.05$).

‡ Significant differences from BZ-1, via analysis of variance ($P < 0.05$).

verapamil-sensitive BZ-1 cells demonstrated a severely depressed $\dot{V}_{max}$ (<20 V/sec), accompanied by partial depolarization (Table I). In contrast, action potentials of the other border zone cells (BZ-2), as well as those recorded from normal and central infarct zones cells, were never abolished even at higher concentrations of verapamil. Action potentials of BZ-2 cells were characterized by abbreviated durations (both APD_{50} and APD_{90}) and moderate depression in $\dot{V}_{max}$ (Table I). During exposure to 1.0 μg/ml verapamil, only action potential amplitude (78 to 71 mV, −9%, n = 4, $P < 0.05$) and duration (APD_{50}, 47 to 35 msec, −26%; APD_{90}, 96 to 69 msec, −28%, n = 4, $P < 0.05$) were significantly altered in BZ-2 cells.

A lower concentration of verapamil (0.1 μg/ml) significantly depressed $\dot{V}_{max}$ (13 to 3 V/sec, −77%, n = 3, $P < 0.05$), action potential amplitude (59 to 33 mV, −44%, n = 3, $P < 0.05$), and shortened action potential duration (APD_{50}, 66 to 49 msec, −26%; APD_{90}, 134 to 109 msec, −19%, n = 3, $P < 0.05$) of the slowly rising action potentials of BZ-1 cells (Fig. 1a). In contrast, neither $\dot{V}_{max}$ nor action potential amplitude of BZ-2 cells was affected by 0.1 μg/ml verapamil; this drug concentration only further abbreviated the short action potential duration of BZ-2 cells (APD_{50}, 41 to 32 msec, −22%; APD_{90}, 82 to 67 msec, −18%, n = 3, $P < 0.05$) (Fig. 1b).

Verapamil (0.1 and 1.0 μg/ml) had no effect on action potentials of cells in uninfarcted areas. However, action potential amplitude and APD_{50} of such normal zone cells were significantly reduced by 2.0 μg/ml verapamil (102 to 98 mV, −4%, 90 to 77 msec, − 14%, n = 6, $P < 0.05$) (Fig. 2a). As in the normal zone cells, action potentials of central infarct zone cells were not affected by 0.1 μg/ml verapamil. However, 1.0 μg/ml verapamil significantly shortened APD_{50} of central infarct zone cells by 21% (122 to 96 msec, n = 4, $P < 0.05$). In addition to the reduction of action potential amplitude (109 to 100 mV, −9%, n = 6, $P < 0.05$) and shortening of APD_{50} (113 to 74 msec, −35%, n = 6, $P < 0.05$), 2.0 μg/ml verapamil significantly shortened APD_{90} (181 to 141 msec, −22%, n = 6, $P < 0.05$) of central infarct zone cells (Fig. 2b).

Discussion. The absence of a significant verapamil effect on $\dot{V}_{max}$ of normal and central infarct zones ventricular muscle cells is in keeping with its minimum effect

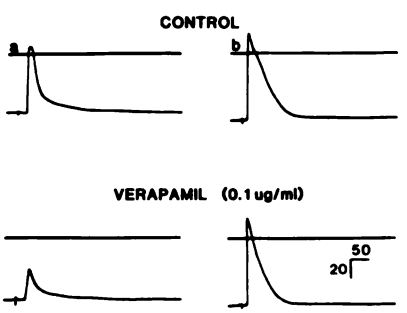

FIG. 1. The effects of 0.1 μg/ml verapamil on the action potential characteristics of ventricular muscle cells from BZ-1 (A) and BZ-2 (B) cells in the border zone. After 60 min superfusion with verapamil, the slowly rising action potential of BZ-1 cell was severely depressed and remained depressed despite increased stimulus intensity (10×), 0.5 mm from the recording site. In contrast, this concentration of drug only abbreviated the already short action potential duration of BZ-2 cell.

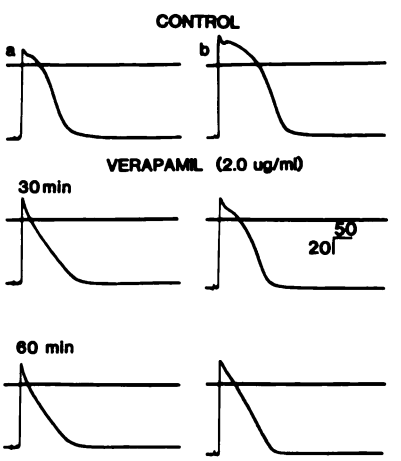

FIG. 2. The effects of 2.0 μg/ml verapamil on the action potential characteristics of ventricular muscle cells from the normal zone (A) and central infarct zone (B). After 30 and 60 min superfusion with verapamil, action potential amplitude and APD_{50} of both normal and central infarct zones cells were significantly reduced. Note the additional shortening of APD_{90} by verapamil monitored in central infarct zone cell.

on the fast Na^+ current in cat myocardium (5). This is consistent with previous reports that verapamil did not change $\dot{V}_{max}$ of normal ventricular cells (3, 12). We also observed that verapamil produced a significant decrease in action potential amplitude from normal and central infarct zones cells. A similar reduction in action potential amplitude by verapamil was documented in normal canine ventricular muscle (13) and it may be that the drug inhibits the Ca^{2+} current which contributes to the action potential overshoot (14).

The shortening APD_{50} of normal and central infarct zone cells and BZ-2 cells by verapamil is consistent with previous reports (15, 16) and can be attributed to a reduction in slow inward plateau current. However, we observed a reduction in APD_{90} of BZ-2 and central infarct zone cells, while that of the normal zone remained unchanged when exposed to verapamil. An alteration of the time-independent plateau current by verapamil (6), in addition to its other effects (a reduction of the time-dependent inward current and a voltage shift of slow outward current), could account for its shortening of APD_{90} of BZ-2 and central infarct zone cells.

The present study shows that in healed infarction myocardium, the electrophysiologic response of muscle cells to verapamil varies even within the border zone. Exposure to verapamil abolished a population of markedly depressed action potentials of cells (BZ-1) in border zones. However, even at higher concentrations of verapamil, action potentials of other border zone cells (BZ-2), as well as that of normal and central infarct zones were never abolished. This suggests that slow inward currents may play an important role in the genesis of action potentials in a border zone population subset. Verapamil-sensitive action potentials have been previously demonstrated in aneurysmal tissues up to 25 months after myocardial infarction in humans (17) and in infarcted tissues 24 hr after coronary ligation in the dog (18). However, in our feline model of healed myocardial infarction, verapamil-sensitive action potentials were most evident in the border zone.

The lack of verapamil effect on $\dot{V}_{max}$ and the persistence of electrical activity of BZ-2 cells in the presence of verapamil suggests that these action potentials were not slow channel dependent. Although a defect in the conductance of the fast Na^+ current is a possible mechanism, we cannot be sure of the ionic nature of the slightly depressed action potentials of BZ-2 cells.

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1. Wong SS, Bassett AL, Epstein K, Kozlovskis P, Ezrin AM, Silver J, Myerburg RJ. Border zone and infarct zone cells display different electrophysiologic abnormalities after healing of myocardial infarction. Circulation (Suppl. IV) 64:318, 1981.
2. Beeler GW, Reuter H. Voltage clamp experiments on ventricular fibers. J. Physiol. (London) 207:165–190, 1970.
3. Cranefield PF, Aronson RS, Wit AL. Effect of verapamil on the normal action potential and on a calcium-dependent slow response of canine Purkinje fibers. Circ Res 34:204–213, 1974.
4. Chen CM, Gettes LS. Effects of verapamil on rapid Na channel-dependent action potentials of K^+-depolarized ventricular fibers. J Pharmacol Exp Ther 209:415–421, 1979.
5. Kohlhardt M, Bauer B, Krause H, Fleckenstein A. Differentiation of the transmembrane Na and Ca channels in mammalian cardiac fibres by the use of specific inhibitors. Pflügers Arch 335: 309–322, 1972.
6. Kass RS, Tsien RW. Multiple effects of calcium antagonists on plateau currents in cardiac Purkinje fibers. J Gen Physiol 66:169–192, 1975.
7. Myerburg RJ, Gelband H, Nilsson K, Sung RJ, Thurer RJ, Morales AR, Bassett AL. Long-term electrophysiological abnormalities resulting from experimental myocardial infarction in cats. Circ Res 41:73–84, 1977.
8. Myerburg RJ, Nilsson K, Gelband H. Physiology of canine intraventricular conduction and endocardial excitation. Circ Res 30:217–243, 1972.
9. Bigger JT, Bassett AL, Hoffman BF. Electrophysiological effects of diphenylhydantoin on canine Purkinje fibers. Circ Res 22:221–235, 1968.

10. Steel R, Torrie JH. Principles and Procedures of Statistics. New York, McGraw-Hill, 1960.
11. Dixon WJ, Massey FJ. Introduction to Statistical Analysis. New York, McGraw-Hill, 1969.
12. Rosen MR, Wit AL, Hoffman BF. Electrophysiology and pharmacology of cardiac arrhythmias. VI. Cardiac effects of verapamil. Amer Heart J 89:665-673, 1975.
13. Hirata Y, Kodama I, Iwamura N, Shimizu T, Toyama J, Yamada K. Effects of verapamil on canine Purkinje fibres and ventricular muscle fibres with particular reference to the alternation of action potential duration after a sudden increase in driving rate. Cardiovasc Res 13:1-8, 1979.
14. Mary-Rabine L, Hoffman BF, Rosen MR. Participation of slow inward current in the Purkinje fiber action potential overshoot. Amer J Physiol 237:H204-H212, 1979.
15. Rosen MR, Ilvento JP, Gelband H, Merker C. Effects of verapamil on electrophysiologic properties of canine cardiac Purkinje fibers. J Pharmacol Exp Ther 189:414-422, 1974.
16. Danilo P, Hordof AJ, Reder RF, Rosen MR. Effects of verapamil on electrophysiologic properties of blood superfused cardiac Purkinje fibers. J Pharmacol Exp Ther 213:222-227, 1980.
17. Spear JF, Horowitz LN, Hodess AB, MacVaugh H, Moore EN. Cellular electrophysiology of human myocardial infarction. 1. Abnormalities of cellular activation. Circulation 59:247-256, 1979.
18. Dersham GH, Han J. Actions of verapamil of Purkinje fibers from normal and infarcted heart tissues. J Pharmacol Exp Ther 216:261-264, 1981.

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Androgen Suppression of Circulating Immune Complexes and Enhanced Survival in Murine Malaria (41514)

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Abstract. The role of sex hormones as modulators of autoimmune expression, including immune complex deposition and host survival has received considerable attention. In this study BALB/c female mice received 20 mg of depotestosterone cypionate 5 days before and 5 days after infection with the malarial parasite *Plasmodium berghei*. On Day 15 of the infection, surviving mice were monitored for levels of erythrocytes, hematocrits, absolute parasitemias, and circulating immune complexes (CIC). CIC were determined in serum samples by polyethylene glycol insolubilization and direct measurement by helium–neon laser nephelometry. Treated mice showed a 44% reduction in the quantity of CIC, in contrast to infected controls which received cottonseed oil. Mice given the hormone were afforded protection as evidenced by 100% survival on Day 15 of the infection, versus 68% survival for the infected control group. The circulating erythroid and parasitemic levels did not differ significantly between experimental and control groups. We conclude that androgens suppress CIC levels during malaria and further suggest their involvement in the differential host survival noted herein.

Immune complexes have been responsible for a number of immunopathological effects in a variety of diseases, including malaria (1, 2). An immune complex-mediated nephritis may occur in humans (3) and circulating immune complexes (CIC), cryoglobulinemia, and complement consumption have been associated with cerebral malaria (4). In experimental murine malaria, immune complex deposits have been found in the kidneys (5), lungs (6), and choroid plexus (7). The sequential detection of soluble malarial antigens, malarial antibodies, C3 consumption, and CIC have been noted in *Plasmodium berghei* infections (7). Immune complex levels, determined during the course of *P. berghei* infections of several inbred mouse strains, did not relate to numbers of red cells parasitized and observed differences in mortality patterns could not be correlated solely to levels of parasitemia, antibody, or any single serological factor in this study (8). In this regard, CIC and cryoglobulin levels in *Plasmodium falciparum* infections were high in cerebral malaria patients, and low or negligible in uncomplicated or benign malaria (4).

The role of sex hormones in both normal and abnormal immune responses has received considerable attention over the last few years. For some time it has been known that normal pregnant mice may accumulate immune complexes in their renal glomeruli (9) and possible immune complex formation during human pregnancy has recently been reviewed (10). Female B/W (NZB/NZW F_1) mice exhibit an accelerated expression of autoimmune disease, and subsequent development of a fatal immune complex glomerulonephritis (11). It has been further observed that systemic lupus erythematosus occurs about nine times more frequently in women than in men (12). Recently, modulation of the pathological consequences of immune complex disease, including survival, has been demonstrated in a murine lupus model with androgen treatment (13–15) and in experimental autoimmune thyroiditis (16).

This study reports the effect of an androgen primarily on the generation of soluble

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immune complexes, and relates these levels to survival in virulent murine malaria.

Materials and Methods. *Mice.* Twelve-week-old virgin female, inbred BALB/c mice (Charles River Labs, Wilmington, Mass.) were used throughout this investigation. Mice were allowed food and water *ad libitum* until fasted 12 hr before sacrifice.

Malaria infection. Plasmodium berghei berghei, strain NK 65, was maintained by weekly blood passage. Experimental hormone-treated mice, and infected control mice, received an intraperitoneal inoculum containing 5×10^4 parasitized red blood cells. On Day 15 of the infection, mice were tail-bled just prior to exsanguination, and percentage parasitemias, hematocrits, and erythrocyte counts were determined. Serum samples were collected at 4° and normally employed within 24 hr.

Drug treatment. Depotestosterone cypionate (Upjohn, Kalamazoo, Mich.) in cottonseed oil was injected intraperitoneally in a volume of 0.2 ml (20 mg) into mice on two occasions; namely 5 days before and 5 days after infection. Control mice received comparable injections of 0.2 ml cottonseed oil 5 days before and 5 days after infection.

Circulating immune complexes (CIC). The method of Schultz-Ellison *et al.* (17) involving polyethylene glycol insolubilization of CIC and direct measurement by laser nephelometry was performed as described, with the following minor modifications. Twenty-five microliters of a serum sample was combined with 1.0 ml of a pH 8.3 borate buffer containing 3% PEG 6000 (Fisher Scientific Co.) and allowed to incubate for 1 hr at room temperature. A Hyland Laser Nephelometer (Hyland, Costa Mesa, Calif.) incorporating a helium–neon laser light source (6328 Å) was employed to detect the insolubilized complexes. The instrument was used with a photometer blank subtract of medium, and a computing time of 15 sec. A high reference cuvette was used to set the range to 100% relative light scatter (RLS) at a sensitivity of one. The reference cuvette contained a 1:1000 dilution of latex particles, particle size 0.81 μm (Difco Lab., Detroit, Mich.). The percentage relative light scatter (RLS) displayed for each sample was the amount of light scatter produced by the sample, relative to the reference cuvettes. Cohn Fraction II (Sigma Chemical Co., St. Louis, Mo.) was heated to 63° for 20 min, whereupon large aggregates were removed by centrifugation at 2000*g* for 10 min. The concentration of aggregated IgG was determined by absorbance at 280 nm. Twenty-five micrograms of aggregated IgG, combined with 1.0 ml of 3% PEG in borate, generates 12.5% RLS at this setting.

Statistical analyses. Parameters were expressed as the group mean ± one standard error. Based on the nonparametric Mann–Whitney *U* test, *P* values <0.05 were considered to be statistically significant.

Results. Mice receiving testosterone exhibited significantly reduced levels of circulating immune complexes on Day 15 of a virulent murine malaria infection as compared to untreated infected control animals (Table I). Specifically, treated mice showed a 44% reduction in the quantity of soluble complexes, relative to infected mice receiving only cottonseed oil. Treated mice were afforded protection by the hormone as evidenced by 100% survival on Day 15 of the infection (Table II). The circulating erythroid and parasitemic levels did not differ significantly between hormone-treated and infected control mice (Table III).

Discussion. Administration of testosterone, before and after infection with *Plasmodium berghei,* reduced levels of cir-

TABLE I. EFFECT OF TESTOSTERONE ON CIRCULATING IMMUNE COMPLEX (CIC) LEVELS ON DAY 15 OF MALARIAL INFECTION

Groups	Nos. of animals	RLS[a]
Uninfected controls	11	7.4 ± 0.36
Infected controls	20	43.4 ± 2.16
Androgen treated	17	27.5 ± 1.26*

[a] Relative light scatter (%) of polyethylene glycol insolubilized CIC in 25 μl serum samples measured by laser nephelometry. Values represent the group mean ± one standard error.

* Significantly different from uninfected and infected control ($P < 0.05$).

[illegible]

Group	No. of animals	Mean survival	Percentage survival
Normal	[illegible]	[illegible]	[illegible]
Androgen	[illegible]	[illegible]	[illegible]

[illegible] circulating immune complexes (CIC) in Day 15 of the infection as compared to non-treated infected control animals (Table I). Day 15 of the infection was monitored in view of the mortality peaked levels of CIC, degree of parasitemia, and anemia previously observed for this critical period. Reduced levels of CIC are perhaps not too surprising since there is increasing evidence that androgens may suppress both immunoglobulin production and cellular response to immunization (14). Furthermore, androgen-treated malarial mice exhibiting reduced levels of soluble complexes showed enhanced host survival (Table II). Increased survival has been demonstrated in an androgen-treated murine lupus model, along with the reduction of antinuclear and anti-T lymphocyte antibodies and a decreased deposition of immune complexes in the glomerulus (15, 16). Kenshimoto et al. (13) suggest that protection may be due, in part, to androgen

TABLE III. EFFECT OF TESTOSTERONE ON THE ANEMIA AND PARASITEMIA EXHIBITED ON DAY 15 OF MALARIAL INFECTION

Parameters[a]	
Erythrocytes (× 10⁶/mm³)	
Infected controls	3.93 ± 0.27
Androgen treated	3.24 ± 0.18
Hematocrit (%)	
Infected controls	22.6 ± 3.29
Androgen treated	24.6 ± 1.63
Parasitized erythrocytes (%)	
Infected controls	35.2 ± 4.23
Androgen treated	42.4 ± 1.91
Parasitized erythrocytes (× 10⁶/mm³)	
Infected controls	1.40 ± 0.21
Androgen treated	1.34 ± 0.08

[a] Each value represents the groups mean ± one standard error of 8–10 animals.

promotion of suppressor T-cell function. In this regard, during the course of systemic lupus erythematosus, both immune complexes and lymphocytotoxic autoantibodies may be responsible for the suppressor T-cell defects noted (18). Concomitant autocytotoxins (19) and immune complexes may play a role in the observed T-cell deficit in murine malaria as well (20).

We have previously demonstrated that overcoming the anemia associated with murine malaria by red cell hypertransfusion is an effective maneuver to promote recovery (21). However, enhanced survival of androgen-treated malarial infected animals is not effected by a reduced anemia or parasitemia, as clearly seen in Table III. The erythropoietic capability of mice infected with malaria is impaired (22) and contributes to the severe anemic status. Although androgens have been shown to stimulate erythropoiesis (23), treated infected mice were essentially as anemic as infected control mice. The only parameter monitored, that was significantly different from infected control mice, was the levels of circulating immune complexes. There is obviously a danger in concluding that CIC were subsequently responsible for the variant host survival seen. Nevertheless the varied immunopathological effects of immune complexes on host systems (2) argue for a significant role in host survival patterns observed during this study. In view of the survival benefit associated with CIC reduction, herein described, it might be of interest to determine the effect of CIC removal by *ex vivo* adsorption, as employed in other studies.

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1. W.H.O. The role of immune complexes in disease. Tech Rep Ser 606, 1977.
2. Theofilopoulos AN, Dixon FJ. The biology and detection of immune complexes. Advan Immunol 28:89–195, 1979.
3. Boonpucknavig V, Sipritja V. Renal disease in acute *Plasmodium falciparum* infection in man. Kidney Int 16:44–52, 1979.
4. Adam C, Geniteau M, Gougerot-Pocidalo M, Ver-

roust P, Lebros J, Gilbert C, Morel-Marager L. Cryoglobulins, circulating immune complexes and complement activation in cerebral malaria. Infect Immun 31:530–535, 1981.
5. Boonpucknavig S, Boonpucknavig V, Bhamarapravati N. Immunological studies of *Plasmodium berghei* infected mice. Arch Pathol 94:322–330, 1972.
6. Weiss ML. Immune complexes in the lungs of mice infected with *Plasmodium berghei*. Isr J Med 14:655–661, 1978.
7. June CH, Contereras CE, Perrin LH, Lambert PH, Miescher PA. Circulating and tissue bound immune complex formation in murine malaria. J Immunol 122:2154–2161, 1979.
8. Contreras CE, June CH, Perrin LH, Lambert PH. Immunopathological aspects of *Plasmodium berghei* infection in five strains of mice. I. Immune complexes and other serological features during the infection. Clin Exp Immunol 42:403–411, 1980.
9. Tung KS. Immune complex in the renal glomerulus during normal pregnancy. A study in the guinea pig and the mouse. J Immunol 11:186–200, 1974.
10. Gleicher N, Theofilopoulos AN. Immune complexes (ICs) and pregnancy. Diagn Gynecol Obstet 2:7, 1980.
11. Howie JB, Heyler BJ. The immunology and pathology of NZB mice. Advan Immunol 9:215–266, 1968.
12. Inman RD. Immunologic sex differences and the female predominance in systemic lupus erythematosus. Arthritis Rheum 21:849–852, 1978.
13. Roubinian JR, Talal N, Greenspan JS, Goodman JR, Siteri PK. Effect of castration and sex hormone treatment on survival, antinucleic acid antibodies and glomerulonephritis in NZB/NZW F_1 mice. J Exp Med 147:1568–1583, 1978.
14. Raveche ES, Tjio JH, Boegel W, Steinberg AD. Studies of the effects of sex hormones on autosomal and X-linked genetic control of induced and spontaneous antibody production. Arthritis Rheum 22:1177–1187, 1979.
15. Verheul HAM, Stimson WH, Pen-Hollander FC, Schurs AH. The effects of nandrolone, testosterone and their decanoate esters on murine lupus. Clin Exp Immunol 44:11–17, 1981.
16. Obayasu I, Kong YC, Rose NR. Effect of castration and sex hormones on experimental autoimmune thyroiditis. Clin Immunol Immunopathol 20:240–245, 1981.
17. Schultz-Ellison G, Charland C, Driscoll J, Thayer W. A rapid method for immune complex detection: PEG insolubilization combined with laser nephelometry. J Immunol Methods 31:31–40, 1979.
18. DeHoratius RJ, Tung KS, Pincus T. Reduced T-lymphocyte subsets in systemic lupus erythematosus: Effects of immune complexes and lymphocytotoxic antibodies. Clin Immunol Immunopathol 17:245–256, 1980.
19. Chung PR, Balsamo E, Gray A, Rencricca NJ, Coleman RM. Autocytotoxins during rodent malaria. J Parasitol 66:847–849, 1980.
20. Brissette WH, Coleman RM, Rencricca NJ. Depressed splenic T lymphocyte numbers and thymocyte migratory patterns in murine malaria. Proc Soc Exp Biol Med 159:317–320, 1978.
21. Hejna JM, Rencricca NJ, Coleman RM. Effective recovery and immunity to virulent malaria following red cell transfusion at crisis. Proc Soc Exp Biol Med 146:462–464, 1974.
22. Rencricca NJ, Coleman RM. Altered erythropoiesis during the course of virulent murine malaria. Proc Soc Exp Biol Med 162:424–428, 1979.
23. Rencricca NJ, Solomon J, Fimian WJ, Howard D, Rizzoli V, Stohlman F. The effect of testosterone on erythropoiesis. Scand J Haematol 6:431–436, 1969.

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Characteristics of Rat Skull Benzylamine Oxidase (41515)

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Abstract. Benzylamine oxidase (BzAO) is an amine oxidase with a widespread distribution in mammalian tissues. The present study has characterized certain properties of BzAO using homogenate of rat skull as the enzyme source. BzAO activity was assayed after inactivation of monoamine oxidases with pargyline and was shown to be distinct from diamine oxidase, polyamine oxidase, lysyl oxidase, and ceruloplasmin. Only aromatic amines with a primary amino group were found to interact with BzAO. 2-Phenylethylamine, tryptamine, *p*-tyramine, and dopamine acted as substrates but were deaminated less rapidly than benzylamine, which showed an apparent K_m value of 2.8 μM and a V_{max} value of 220 pmole deaminated mg·protein^{-1}·min^{-1}. (+)-α-Methylphenylethylamine (amphetamine) acted as a noncompetitive inhibitor of benzylamine deamination. Overall, the K_m and K_i values of the amines for BzAO increased with increasing polarity. It is concluded that the nonprotonated form of benzylamine acts as substrate for the enzyme and that the catalytic mechanism of skull BzAO appears consistent with a double displacement or "ping-pong" reaction. Compared with brain monoamine oxidase type B, benzylamine is 50 times more avid for BzAO, which, in turn, appears to exhibit a lower K_m for oxygen than monoamine oxidase type B.

Benzylamine oxidase (BzAO) is an amine oxidase present in several species including man but, to date, its physiological function is unknown. Although benzylamine is oxidatively deaminated by BzAO it is also a substrate for monoamine oxidase (MAO; EC 1.4.3.4., monoamine: O_2 oxidoreductase deaminating), particularly the B-type (1–4). Unlike MAO, however, which is inhibited by pargyline, clorgyline, and deprenyl, BzAO is inhibited by carbonyl-trapping agents such as semicarbazide. Thus, differential assay of BzAO and MAO is possible utilizing the appropriate inhibitors (2, 3, 5, 6).

BzAO was first discovered in plasma (7) and since then the plasma enzyme has been purified and characterized (1, 8–11). More recently, BzAO activity has been found to be widespread in the peripheral organs of rat and man (2–6) but this BzAO has not received the detailed attention afforded the plasma enzyme. For instance, the substrate profile of the enzyme in cellular tissues has not been defined fully and differentiation of BzAO from amine oxidases other than MAO, and which also deaminate benzylamine, has not been complete. We have shown recently that bones of the rat skull contain good BzAO activity (6). Therefore, the present study was undertaken to define and characterize skull BzAO with regard to substrate specificity, kinetic constants, pH influences, and oxygen dependence. In the latter experiments, BzAO was compared with MAO type B obtained from rat brain.

Materials and Methods. Male Sprague–Dawley rats (TIMCO Breeding Laboratories, Houston, Tex.) weighing 150–250 g were used. The rats were given free access to food and water and were housed in air-conditioned quarters on a 12-hr light–dark cycle.

Preparation of tissues. Rats were killed by decapitation. The skull (carefully scraped free of all adhering tissue) and brain were rinsed thoroughly in saline (0.9% NaCl, w/v), blotted dry, and weighed. The skull was ground up with a mortar and pestle while the brain was minced with scissors. The tissues were then homogenized (Ultra Turrex, Model SDT, for 1 min at a setting of 4.5) using a tissue: buffer ratio of 1:10 in 1 mM phosphate buffer, pH 7.8. The ho-

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mogenates were centrifuged at 700*g* for 10 min and supernatant fractions of 1 or 2 ml were frozen at −25° in glass vials for subsequent assay. All preparative procedures were done at 4°.

Enzyme assays. BzAO and MAO-B activities were determined radiochemically by the method of McCaman *et al.* (12) as modified by Callingham and Laverty (13). BzAO activity was measured after preincubating 25 μl homogenate with 25 μl pargyline (4×10^{-4} *M* for 20 min at 37°) to inhibit MAO activity. Semicarbazide (4×10^{-4} *M*) was used in place of pargyline to measure MAO activity. Following the appropriate preincubation, 50 μl of the radiolabeled amine (freshly prepared in 0.2 *M* phosphate buffer, pH 7.8) was added and each reaction was run, in triplicate, for 15 min at 37°. Enzyme activity was stopped by cooling the tubes on ice followed by the addition of 10 μl 3 *N* HCl. Blank values were obtained by adding the acid just prior to the addition of the labeled amine and represented less than 3% of the test values. Deaminated products were extracted in 600 μl of toluene:ethylacetate (1:1, v/v, saturated with water) and a 400-μl aliquot taken for liquid scintillation counting with quench correction. The efficiency of extraction for the various deaminated metabolites ranged from 80% (dopamine) to 98% (benzylamine) and all results are corrected for recovery.

Potential substrates and inhibitors. Various amines with a potential for substrate capability were added to the assay tubes at the same time as benzylamine (K_m concentration) whereas potential inhibitors were preincubated for 20 min at 37° prior to the addition of substrate. Control tubes contained appropriate amounts of water in place of the added amine or inhibitor and the enzymatic reaction was run for 15 min, as described above. In order to maximize inhibition of benzylamine deamination all compounds were tested at a concentration of 1 m*M*. None of the added substances interfered with the extraction of the deaminated metabolites.

Kinetic studies. The initial velocity rates were measured after a 5-min incubation at 37°. With BzAO, the final concentrations of the substrates were: 1, 1.25, 1.67, 2.5, 5, 10, and 20 μ*M* for benzylamine; 3, 4.35, 6.67, 13.33, 40, and 166.67 μ*M* for both 2-phenylethylamine and dopamine; 16.7, 25, 33.33, 50, 66.67, and 100 μ*M* for both tryptamine and *p*-tyramine. With MAO, 100, 167, 250, 500, and 800 μ*M* benzylamine was required. The apparent K_m and V_{max} values were calculated by computer program according to the method of Wilkinson (14).

K_i values were determined by adding the unlabeled amine at the same time as labeled benzylamine. The final concentrations of the unlabeled amines were: 7.5, 15, and 30 μ*M* for 2-phenylethylamine and 25, 50, and 100 μ*M* for both tryptamine and (+)-amphetamine.

Oxygen tensions were altered in some kinetic experiments by bubbling the assay tubes for 15 sec at 4° with either nitrogen or oxygen and quickly sealing the tubes with rubber stoppers.

Effect of pH. The pH of the final assay mixture was varied between 5 and 10 by adjusting the pH of the 0.2 *M* phosphate buffer.

Protein. The protein content of homogenates was measured by the microbiuret method of Goa (15) with bovine serum albumin (Fraction V) as the standard.

Chemicals. The following radiolabeled compounds were used: [^{14}C]benzylamine HCl (12.5 mCi·mmole^{-1}); [^{3}H]tryptamine HCl (0.71 Ci·mmole^{-1}); [^{14}C]dopamine HCl (44.8 mCi·mmole^{-1}); 2-[^{14}C]phenylethylamine HCl (52.0 mCi·mmole^{-1}) and [^{14}C]-tyramine HCl (50.7 mCi·mmole^{-1}). The specific activities and concentrations of these compounds were adjusted using the nonlabeled hydrochloride salts of the respective amines. All other compounds and chemicals were of analytical grade where possible.

Results. Table I shows the effect of various compounds on the deamination of benzylamine in homogenate of rat skull. Monoamine oxidases were inactivated previously by preincubation with pargyline (see Materials and Methods). Overall, 23 different compounds were tested including derivatives of 2-phenylethylamine, indoleamines, diamines, polyamines and

TABLE I. EFFECT OF VARIOUS COMPOUNDS ON THE DEAMINATION OF BENZYLAMINE BY BENZYLAMINE OXIDASE[a]

Compound	Percentage control activity	Compound	Percentage control activity
Benzylamine	1.2	Histamine	55.0
2-Phenylethylamine	2.6	Lysine vasopressin	82.1
Phenylethanolamine	3.0	*N*-Methylphenyl-ethanolamine	86.5
(+)-Amphetamine	6.4	*N*-Methylphenyl-ethylamine	97.1
N-(1-naphthyl)ethylene-diamine	6.9	Putrescine	102.0
Tyramine	10.1	Cadaverine	108.0
Tryptamine	13.1	KCN	109.0
Dopamine	17.2	(±)-Penicillamine	110.0
5-Hydroxytryptamine	25.4	Spermine	115.0
Normetanephrine	28.8	Spermidine	115.0
N,N-dimethyl-*p*-phenylenediamine	29.0	γ-Aminobutyric acid	118.0
Norepinephrine	36.1		

[a] Homogenate was preincubated for 30 min at 37° with pargyline to inactivate monoamine oxidase. KCN and (±)-penicillamine were then added and preincubated for a further 30 min at 37°. Other compounds were added at the same time as [^{14}C]benzylamine (3 μ*M*) and the reaction was run for 15 min at 37° using triplicate determinations. All test compounds were used at 1 m*M*.

other miscellaneous substances. Only amines containing a primary amine group caused marked inhibition of benzylamine deamination. Thus, 2-phenylethylamine was found to be a very good inhibitor whereas *N*-methyl-phenylethylamine was inactive. In general, inhibitory activity of the phenylethylamines decreased as polarity increased. The aliphatic straight chain diamines (cadaverine and putrescine) and the aliphatic straight chain polyamines (spermine and spermidine) were inactive at inhibiting benzylamine deamination. Peptidyl-bound lysine (lysine vasopressin) failed to give marked inhibition and it is noteworthy that the enzymatic activity was insensitive to (±)-penicillamine and potassium cyanide.

Certain aromatic monoamines were selected to test for substrate capability and kinetic analysis. Table II shows that 2-phenylethylamine, certain of its derivatives, and the indoleamine, tryptamine, acted as substrates for rat skull BzAO. However, it is clear that benzylamine is the preferred substrate. Ring hydroxylation, as with *p*-tyramine and dopamine, gave increased K_m values. 2-Phenylethylamine and

TABLE II. KINETIC CONSTANTS OF VARIOUS AMINES FOR BENZYLAMINE OXIDASE[a]

Amine	K_m (μ*M* ± SE)	V_{max} (pmole deaminated·mg protein^{-1}·min^{-1} ± SE)	Interaction with benzylamine	K_i (μ*M*)
Benzylamine	2.8 ± 0.2	220.2 ± 0.1	—	—
2-Phenylethylamine	14.5 ± 1.1	47.8 ± 1.8	Competitive	14.2
Tryptamine	54.3 ± 7.4	57.2 ± 4.2	Competitive	55.0
p-Tyramine	66.8 ± 7.6	36.6 ± 2.3	—	—
Dopamine	269.7 ± 10.1	80.7 ± 2.0	—	—
(+)-Amphetamine	—	—	Noncompetitive	35.5

[a] All experiments were done at pH 7.8 under an atmosphere of air, using triplicate determinations at each substrate concentration used. The K_m and V_{max} values for dopamine must be regarded as approximations since the concentrations of dopamine employed were below those required for valid estimates (see Materials and Methods). The K_i value for (+)-amphetamine is likely to represent a hybrid value resulting from an interaction with both the oxidized and reduced forms of BzAO (see Discussion).

tryptamine were shown to act as competitive inhibitors of benzylamine deamination and the resulting K_i values agree well with the K_m determinations. (+)-Amphetamine (alpha-methyl-phenylethylamine) acted as a noncompetitive inhibitor with an inhibition constant consistent with its low polarity.

The effect of varying pH on the kinetic constants for benzylamine was determined at pH 6.9, 7.3, 7.8, and 8.2. Full double reciprocal plots were done at each pH and the K_m and V_{max} values computed. The results are illustrated in Fig. 1. Over the pH range studied, the apparent K_m values declined almost linearly as pH increased. It was noted, however, that calculation of the pH-independent K_m values (the K_m in terms of the concentration of nonprotonated benzylamine) showed little deviation from 0.05 μM (Fig. 1). The pH-independent K_m values were computed from McEwen's (16) modification of the Henderson–Hasselback equation by substituting in the experimentally derived K_m values and using 9.37 as the pK_a for benzylamine. The V_{max} values at pH 6.9 and 7.3 were comparable but diminished rates were obtained at the two higher pH values. In another experiment (Fig. 2), the deamination of a single concentration of benzylamine (50 μM) was studied. Maximal deamination was found at pH 7.4.

The results of altered oxygen tensions on the deamination of benzylamine by BzAO are shown in Fig. 3 and Table III. For comparison, a similar experiment was done with MAO from rat brain. We have shown previously that the deamination of benzylamine by rat brain homogenate is due entirely to MAO type B (6). Under an at-

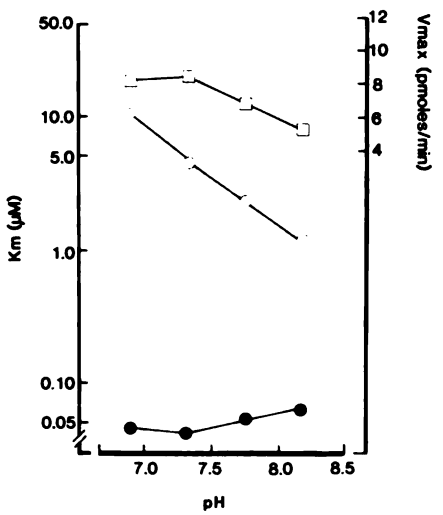

FIG. 1. Effect of pH on the Michaelis–Menten constants of benzylamine for benzylamine oxidase. Ordinate, left-hand side: experimentally derived K_m values (○) and calculated pH-independent K_m values (●) on a log scale. Ordinate, right-hand side: V_{max} values (□). Abscissa: pH of the reaction mixture. The values were derived from full double reciprocal plots using triplicate determinations at each benzylamine concentration. The enzyme reaction was run for 5 min after inactivation of monoamine oxidase with pargyline. The pH-independent K_m values (the K_m in terms of the concentration of nonprotonated benzylamine) were computed from McEwen's (17) modification of the Henderson–Hasselback equation using the experimentally derived K_m values and 9.37 as the pK_a for benzylamine.

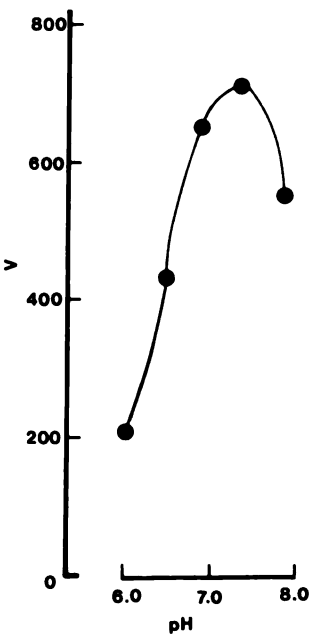

FIG. 2. pH optima curve for benzylamine deamination by benzylamine oxidase. Ordinate: v, velocity of the enzyme reaction. Abscissa: pH of the reaction mixture. Benzylamine oxidase activity was assayed using 50 μM benzylamine for 15 min at 37° after inactivation of monoamine oxidase with pargyline. Each point is the mean of triplicate determinations.

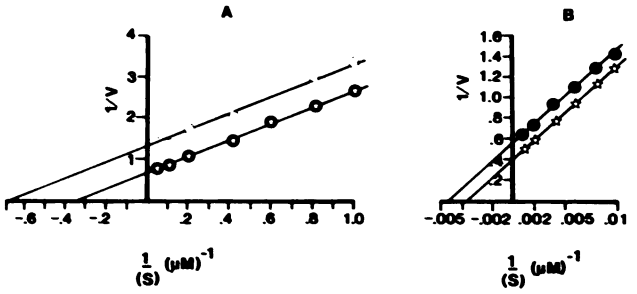

FIG. 3. Double reciprocal plots for benzylamine deamination by benzylamine oxidase (A) and monoamine oxidase type B (B) in the presence of differing oxygen tensions. Ordinate: reciprocal of velocity (v) of the enzyme reaction in arbitrary units. Abscissa: reciprocal of the benzylamine concentration. The enzyme reactions were run for 5 min and each point is the mean of triplicate determinations done under an atmosphere of air (●), oxygen (☆), or nitrogen (○) at pH 7.8. Note that the double reciprocal plots for benzylamine oxidase, whether assayed under air or oxygen, are identical. For kinetic constants, see Table III and for further experimental details, see Materials and Methods.

mosphere of oxygen the double reciprocal plot for BzAO was identical to that obtained under air. In contrast, a parallel, uncompetitive shift was obtained for MAO type B. For BzAO, oxygen dependence only became evident after flushing the assay tubes with nitrogen. The apparent K_m and V_{max} values were then decreased by 50% (Table III). It is clear from Table III that BzAO appears as a high-affinity, low-capacity enzyme for benzylamine deamination, compared with MAO type B.

Discussion. Recently we reported BzAO activity in the bones of rat skull (6) and the present study has investigated certain properties of this amine oxidase.

TABLE III. APPARENT KINETIC CONSTANTS FOR BENZYLAMINE DEAMINATION BY BENZYLAMINE OXIDASE (BzAO) AND MONOAMINE OXIDASE TYPE B (MAO-B) UNDER DIFFERING OXYGEN TENSIONS*

Tissue and condition	K_m (μM)	V_{max} (pmole deaminated · mg protein⁻¹ · min⁻¹)
BzAO (skull)		
Oxygen	3.0	223
Air	3.0	223
Nitrogen	1.5	113
MAO-B (brain)		
Oxygen	223.5	3630
Air	158.5	2620

* For double reciprocal plots, see Fig. 2.

The results shown in Table I give important information concerning the characteristics and definition of BzAO. The enzyme displays characteristics typical of pyridoxal-dependent amine oxidases (1) in that a primary amine group is required for interaction. The inability of the aliphatic diamines (putrescine and cadaverine) and the aliphatic polyamines (spermine and spermidine) to inhibit benzylamine deamination clearly distinguish BzAO from diamine oxidase and polyamine oxidase (spermine oxidase). An indication for substrate overlap with diamine oxidase is suggested from the inhibitory action of histamine (45%) and with ceruloplasmin from the marked inhibition with *N,N*-dimethyl-*p*-phenylenediamine (71%). However, the involvement of ceruloplasmin is ruled out by insensitivity to potassium cyanide (17). BzAO is also quite distinct from lysyl oxidases since it is resistant to inhibition by (±)-penicillamine and was only slightly affected by lysyl vasopressin. In addition, according to Shieh *et al.* (18), benzylamine is not a substrate for lysyl oxidase. Thus, overall, there is little doubt that BzAO of rat skull represents a separate and distinct activity.

Of the amines tested for substrate capability, it is obvious that benzylamine acts as the preferred substrate (Table II). Skull BzAO, however, is not without catalytic activity upon the indoleamine and

phenylethylamine structure with tryptamine and 2-phenylethylamine interacting competitively toward benzylamine. Ring hydroxylation of 2-phenylethylamine, as with *p*-tyramine and dopamine, resulted in higher K_m values indicating a reduced affinity for BzAO. Thus, BzAO resembles MAO type B which also shows a distinct preference for relatively nonpolar amines (benzylamine and 2-phenylethylamine). However, benzylamine possesses a much lower K_m value for BzAO than MAO type B. Under an atmosphere of air, the respective apparent K_m values were about 3 and 158 μM at pH 7.8 (Table III). This is not the case for 2-phenylethylamine which exhibits an apparent K_m value of 3 μM for MAO type B (19) versus 14.5 μM for BzAO (Table I).

The low apparent K_m value of benzylamine for BzAO suggests that this amine might act as the endogenous substrate for the enzyme. Certainly, low concentrations of benzylamine would be deaminated preferentially by BzAO rather than by MAO type B. So far, however, benzylamine has not been detected in the tissues of animals or man, nor is it present in the atmosphere. On the other hand, the V_{max} values for the endogenously occurring amines (Table II) are so low that deamination by BzAO would be predicted to be negligible compared with that resulting from MAO, an enzyme which consistently exhibits V_{max} values in the nanomolar per minute range, even with benzylamine as substrate (Table III). It is not known whether this difference reflects turnover rates of the respective enzymes or merely differences in enzyme concentration. Also, with intact cells, *in vivo*, it is possible that the access of amines to BzAO versus MAO may differ, resulting in a relatively greater metabolic role of BzAO than would be predicted from *in vitro* studies. Recently, BzAO has been reported to be located in the plasma membrane of cells (20) and as such it would be much more readily available to plasma-borne substrates than mitochondrial MAO. In fact, in the isolated perfused rabbit lung, Roth and Gillis (21) found that 30% of 2-phenylethylamine deamination proceeded via BzAO using a perfusion concentration (1·1 μM) which should favor metabolism by MAO. It remains to be established, however, whether the rabbit lung enzyme possesses the same properties as those reported here for rat skull BzAO.

The substrate profile of skull BzAO seems similar to that of peripheral rat blood vessels (2, 22) and the plasma BzAO of several species, including man (1, 7–9, 23). However, it is unlikely that the activity in skull results from contamination with blood since rat serum (2) and whole blood (21) are virtually devoid of BzAO activity. It is of interest to note that in the organs of man, BzAO does not act upon 2-phenylethylamine and dopamine (2). Furthermore, the apparent K_m value for benzylamine is about 40 times higher than that reported here (2). Thus, the rat enzyme may not serve as a reliable model for the BzAO of man.

Evidence exists that pig (11) and human (16) plasma BzAO acts upon the nonprotonated form of the amine substrate although contradictory conclusions have been drawn in other species (25, 26). In the present study, kinetic experiments done with benzylamine between pH 6.9 and 8.2 gave apparent K_m values ranging from 11.0 to 1.1 μM. However, when these experimentally derived K_m values were calculated with respect to the concentration of nonprotonated benzylamine, a relatively constant, pH-independent value of 0.05 μM was obtained (Fig. 1). This result suggests strongly that the nonprotonated form of benzylamine acts as the substrate for rat skull BzAO and that ionizable binding sites on the enzyme are not altered radically between pH 6.9 and 8.2. For comparison, the pH-independent K_m values for 2-phenylethylamine and tryptamine were calculated to be 0.15 and 0.22 μM, respectively. Thus, benzylamine remains as the most highly preferred substrate, although on a pH-independent basis, its K_m value is now only three times less than that of 2-phenylethylamine (compare with Table II).

From the present experiments, it is not clear whether the effect of pH on product formation (Figs. 1 and 2) is due to a direct

influence on catalytic activity and/or whether it results from effects on substrate ionization. Progressive saturation of BzAO up to the pH optimum of 7.4, followed by substrate inhibition, might explain the data. That BzAO is subject to substrate inhibition has been noted previously (2, 6). This interpretation, however, although attractive, remains to be proven.

From our kinetic studies (Fig. 3) it appears that BzAO possesses a lower K_m for oxygen than brain MAO-B. Whereas an increase in the oxygen tension of the reaction mixture produced a parallel, uncompetitive shift in the double reciprocal plot for MAO-B, it was without effect on BzAO. Oxygen dependence for BzAO only became evident after lowering the oxygen tension by flushing with nitrogen. Thus, it may be argued that the oxygen concentration of the reaction mixture under an atmosphere of air is near to or even at saturation for BzAO but not MAO-B. Following flushing with nitrogen, the K_m and V_{max} values decreased by 50% due to an uncompetitive shift in the double reciprocal plot. This result indicates, but does not prove, a double displacement or "ping-pong" mechanism of catalysis. The same catalytic mechanism has been reported for plasma BzAO (10, 11, 27, 28), rat heart BzAO (29), and MAO (30). However, the precise nature of the presumed "ping-pong" reaction for BzAO may differ from that with MAO since (+)-amphetamine was found to be a noncompetitive inhibitor of the reaction (Table II) whereas it acts competitively toward MAO (31). Noncompetitive inhibition in a "ping-pong reaction occurs when an inhibitor combines with both forms of the enzyme. Therefore, the K_i constant for (+)-amphetamine (Table II) is likely to represent a hybrid value resulting from an interaction with both the oxidized and reduced forms of BzAO.

While the present study provides new information concerning certain enzymatic properties of BzAO, the physiological function of this enzyme remains an enigma (23). Studies in man show that the activity of plasma and serum BzAO is reduced in patients suffering from cancer or severe burns (32) while increased activity in plasma has been associated with fibrotic liver disease (9). Whether the BzAO of bone, muscle cells, and other cellular elements is similarly affected is, at present, unknown. The characterization and definition of the enzyme, as described here, should better enable and encourage investigations involving the cellular tissues of rats.

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1. Blaschko H. The natural history of amine oxidases. Rev Physiol Biochem Pharmacol 70:83–148, 1974.
2. Lewinsohn R, Böhm K-H, Glover V, Sandler, M. A benzylamine oxidase distinct from monoamine oxidase B: Widespread distribution in man and rat. Biochem Pharmacol 27:1857–1863, 1978.
3. Lewinsohn R, Glover V, Sandler M. Beta-Phenylethylamine and benzylamine as substrates for human monoamine oxidase A: A source of some anomalies? Biochem Pharmacol 29:777–781, 1980.
4. Lewinsohn R, Glover V, Sandler M. Development of benzylamine oxidase and monoamine oxidase A and B in man. Biochem Pharmacol 29:1221–1230, 1980.
5. Lyles GA, Callingham BA. Evidence for a clorgyline-resistant monoamine metabolizing activity in the rat heart. J Pharm Pharmacol 27:682–691, 1975.
6. Andree TH, Clarke DE. The isolated perfused rat brain preparation in the study of monoamine oxidase and benzylamine oxidase: Lack of selective brain perfusion. Biochem Pharmacol 30:959–965, 1981.
7. Bergeret B, Blaschko H, Hawes R. Occurrence of an amine oxidase in horse serum. Nature (London) 180:1127–1128, 1957.
8. Yasunobu KT, Ishizaki H, Minamiura N. The molecular, mechanistic and immunological properties of amine oxidases. Mol Cell Biochem 13:3–29, 1976.
9. McEwen CM. The soluble monoamine oxidase of human plasma and sera. Advan Biochem Psychopharmacol 5:151–165, 1972.
10. Lindström A, Petterson G. The order of substrate addition and product release during the catalytic action of pig-plasma benzylamine oxidase. Eur J Biochem 84:479–485, 1978.
11. Lindström A, Olsson B, Petterson G. Kinetics of the interaction between pig-plasma benzylamine oxidase and substrate. Eur J Biochem 35:70–77, 1973.

12. McCaman RE, McCaman MW, Hunt JM, Smith MS. Microdetermination of monoamine oxidase and 5-hydroxytryptophan decarboxylase activities in nervous tissue. J Neurochem 12:15–23, 1965.
13. Callingham BA, Laverty R. Studies on the nature of the increased monoamine oxidase activity in the rat heart after adrenalectomy. J Pharm Pharmacol 25:940–947, 1973.
14. Wilkinson GN. Statistical estimations in enzyme kinetics. Biochem J 80:324–332, 1961.
15. Goa J. A microbiuret method for protein determination. Scand J Clin Lab Invest 5:218–222, 1953.
16. McEwen CM. Human plasma monoamine oxidase. II. Kinetic studies. J Biol Chem 240:2011–2018, 1965.
17. Curzon G, Vallet, L. The purification of human ceruloplasmin. Biochem J 74:279–287, 1960.
18. Shieh JJ, Tamaye R, Yasunobu FT. A purification procedure for the isolation of homogenous preparations of bovine aorta amine oxidase and a study of its lysyl oxidase activity. Biochim Biophys Acta 377:229–238, 1975.
19. Dial EJ, Clarke DE. An explanation for deamination of phenylethylamine by multiple types of monoamine oxidase. Pharmacol Res Commun 11:491–500, 1979.
20. Duong AT, Godfraind T, Wibo M. Subcellular localization of two forms of amine oxidase in rat aorta. Brit J Pharmacol 72:510P–511P, 1981.
21. Roth JA, Gillis CN. Multiple forms of amine oxidase in perfused rabbit lung. J Pharmacol Exp Ther 194:537–544, 1975.
22. Coquil JF, Goridis C, Mack G, Neff NH. Monoamine oxidase in rat arteries: Evidence for different forms and selective localization. Brit J Pharmacol 48:590–599, 1973.
23. Buffoni F. Some contributions to the problem of amine oxidases. Pharmacol Res Commun 12:101–114, 1980.
24. Dial EJ, Clarke DE. Phenylethylamine deamination by multiple types of monoamine oxidase. Biochem Pharmacol 27:2374–2375, 1978.
25. Blaschko H, Friedman PJ, Hawes R, Nilsson K. The amine oxidases of mammalian plasma. J Physiol (London) 145:384–404, 1959.
26. McEwen CM, Cullen KT, Sober AJ. Rabbit serum monoamine oxidase, purification and characterization. J Biol Chem 241:4544–4556, 1966.
27. Taylor CE, Taylor RS, Rasmussen C, Knowles PF. A catalytic mechanism for the enzyme benzylamine oxidase from pig plasma. Biochem J 130:713–728, 1972.
28. Yadav KDS, Knowles PF. A catalytic mechanism for benzylamine oxidase from pig plasma. Stopped-flow kinetic studies. Eur J Biochem 114:139–144, 1981.
29. Clarke DE, Lyles GA, Callingham BA. A comparison of cardiac and vascular clogyline-resistant amine oxidase and monoamine oxidase. Inhibition by amphetamine, mexiletine and other drugs. Biochem Pharmacol 31:27–35, 1982.
30. Houslay MD, Tipton KF. The reaction pathway of membrane-bound rat liver mitochondrial monoamine oxidase. Biochem J 135:735–750, 1973.
31. Mantle TJ, Tipton KF, Garrett NJ. Inhibition of monoamine oxidase by amphetamine and related compounds. Biochem Pharmacol 25:2073–2077, 1976.
32. Lewinsohn R. Human serum amine oxidase. Enzyme activity in severely burnt patients and in patients with cancer. Clin Chim Acta 81:247–256, 1977.

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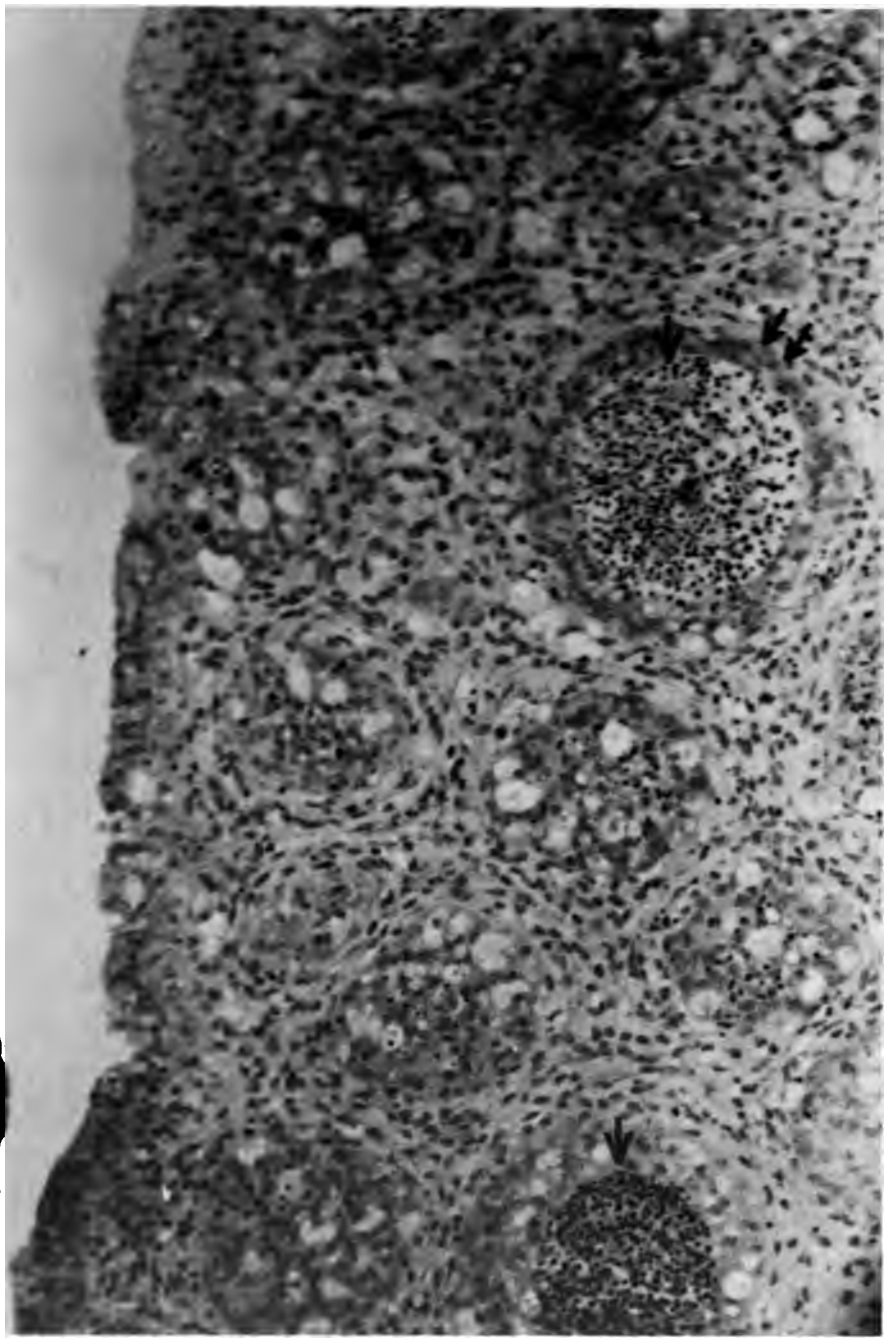


FIG. 2. Bursal fragment after 3 days of culture. Lymphocyte destruction is the prominent feature (single arrows). Basement membrane-associated epithelium is preserved and beginning to proliferate (double arrows) (×100).

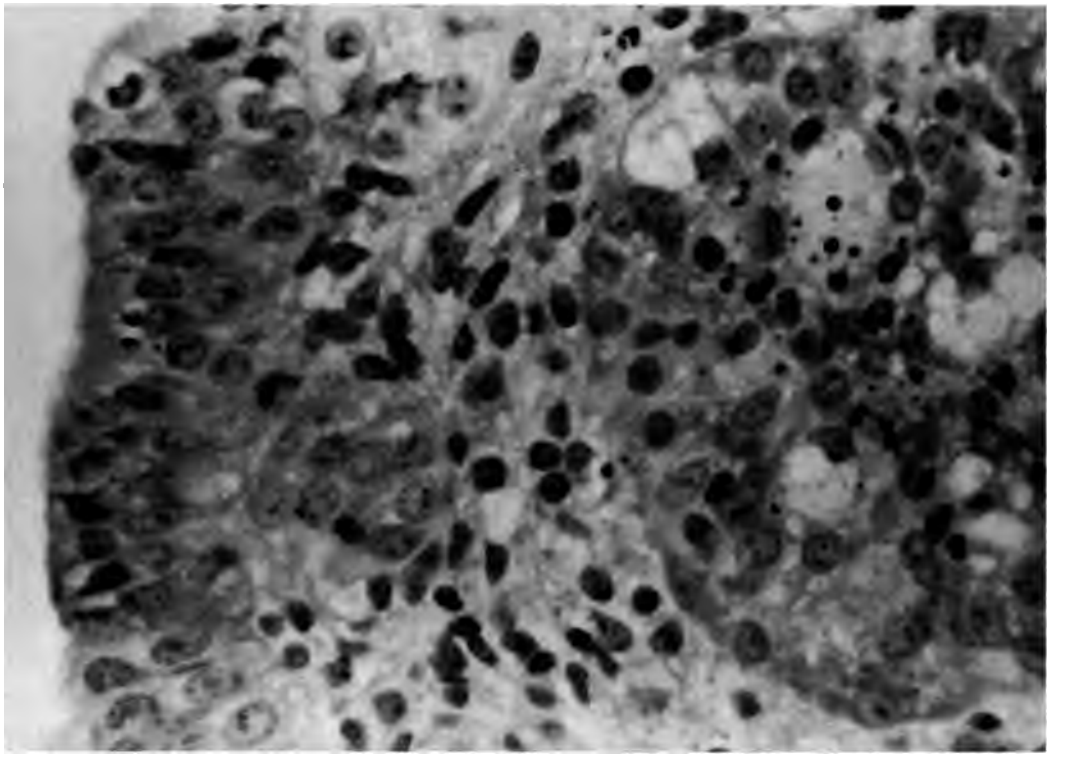


FIG. 3. High-power view of Fig. 2. Nuclear fragments of lymphocytes are seen in the follicle along with epithelial proliferation (×312).

until the grids were submerged and the fluid just touched the bursa pieces. The medium was changed after the first 3 days of culture and again on Day 8. The tissue was incubated in a humidified atmosphere of 5% CO_2 in air at 37° for 13 days.

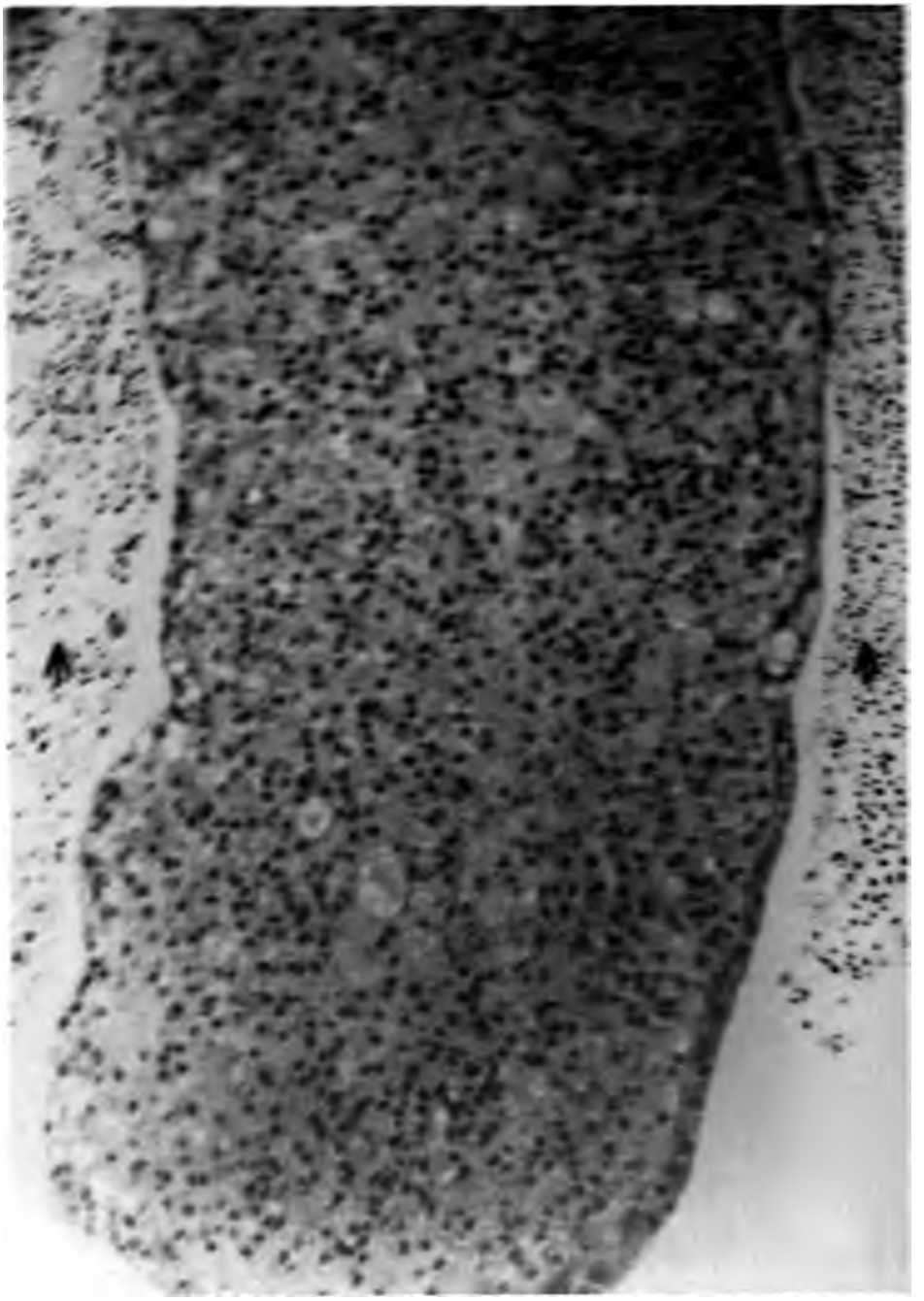
Representative samples of bursal tissue were removed on Days 1, 3, 6, 10, and 13 of culture and frozen or fixed in 10% buffered formalin. Fixed samples were embedded in JB-4 medium (Polysciences, Inc., Warrington, Pa.), sectioned, and stained with hematoxylin and eosin (H&E). Duplicate tissues were stained with an appropriate dilution of rabbit anti-chicken IgG (prepared in our laboratory) followed with FITC-labeled staphylococcal Protein A (Pharmacia Chemical Co., Piscataway, N.Y.). Immunofluorescence was observed using a Zeiss Universal fluorescent microscope with epi-illumination.

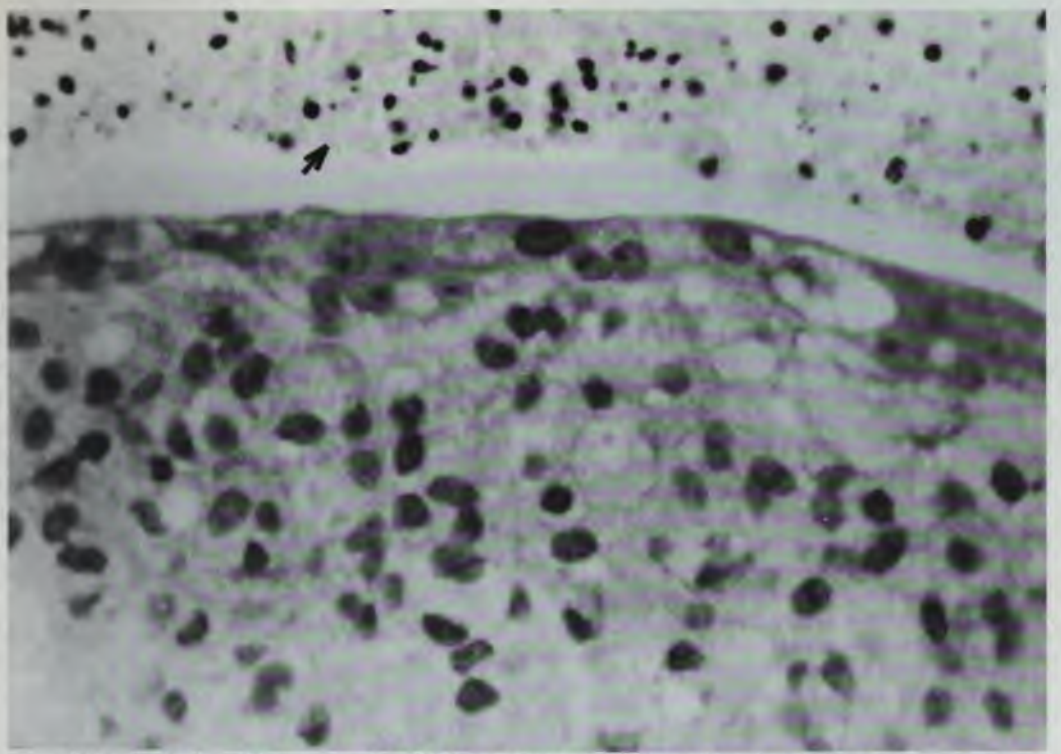
Transplantation. The tissue fragments were removed from culture on Day 13 and pooled in a sterile petri dish containing 5 ml of nonsupplemented RPMI 1640. Meanwhile, the 14-day-old bursectomized chicks were anesthetized with 10–20 μl of methoxyfluorane anesthetic (Pitman-Moore, Inc., Washington Crossing, N.J.), instilled intranasally. A small incision was made in the wing web and 10 pieces of CBE were placed in between the two skin layers forming a pocket. The incision was then sealed with cyanoacrylate adhesive.

Twenty-two days following transplantation, the implants were removed from the wing web and either immersed in liquid nitrogen for frozen sectioning or placed in cold 10% buffered formalin for H&E sections.

The frozen tissue sections were stained with fluorescein-conjugated rabbit anti-chicken γ, μ, and α and examined for the presence of Ig-positive cells.

Results. After 3 days of culture, follicu-





FIGS. 4, 5. Bursal fragment after 10 days culture. Follicular orientation is lost and basement membrane-associated epithelial cells are diffusely layered under plical and specific follicular epithelial layers. Fragment is alymphoid. Emptying of necrotic follicular elements into plical spaces is seen (arrow). (Fig. 4, ×100; Fig. 5, ×312).

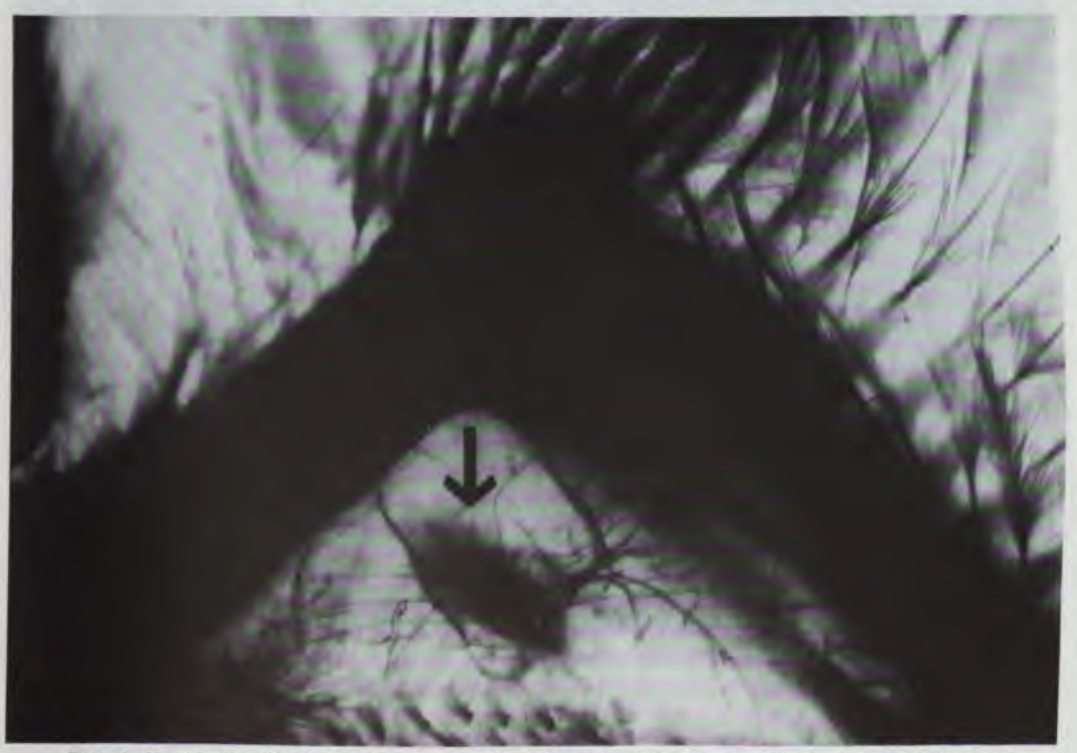


FIG. 6. Fragments of cultured bursal epithelium transplanted 6 days earlier in 14-day-old bursectomized chicks (arrow). Increased vascularity of tissue around transplant is seen.

FIG. 7. Sections of cultured bursal fragments implanted 22 days earlier into wing web of 14 day old chick. Good lymphocyte repopulation with organization into well developed follicle-like structures (arrows) are seen (×63).

lar organization was somewhat preserved, but nearly complete lymphocyte dissolution had occurred. Marked karyorrhexis was apparent, usually in a follicular center, while the basement membrane-associated epithelium remained healthy and appeared to proliferate (Figs. 1, 2, 3). Indirect immunofluorescence indicating the presence of Ig-positive lymphocytes was seen only during the first 36 hr of culture. By Day 10, follicular organization was lost and its epithelium was seen diffusely layered under intact plical or specific follicular layers. Evidence of emptying of the follicular contents into plical spaces as described by Eerola (6) was seen (Figs. 4, 5). Widespread necrosis usually occurred rapidly after Day 14.

On Day 13, the day of transplantation, the fragments were almost totally devoid of lymphocytes. There were no Ig-positive cells seen in the fragments. The bursal epithelium remained intact and appeared viable with no signs of necrosis.

Three to 6 days post-transplantation, the wing web implants in the 14-day-old chicks began to enlarge and become vascularized (Fig. 6). After 22 days, microscopic examination of the transplanted bursal fragments revealed extensive lymphocyte repopulation. There was evidence that follicular organization with corticomedullary demarcation was also reappearing in the implants (Fig. 7). Little necrosis of lymphocytes or parenchymal tissues was observed. In addition, no histological evidence of graft rejection was observed. Previous studies of line 6, subline 1 chickens showed sufficient histocompatibility such that rejection was not anticipated. Stone (7) showed complete acceptance of skin grafts between 42 chickens of this strain. Immunofluorescence revealed collections of Ig-staining cells of all three major classes (Fig. 8).

Discussion. It has been suggested that optimal development of the B-cell repertoire within the bursa is dependent upon close association with the gut environment and its associated antigens (8). Results of our study and others (9) have shown that follicular organization of lymphocytes and appearance of Ig-positive cells within a bursal epithelium can occur at a site other than the gut. In this study, the wing web proved to be an ideal site for both the initial implantation of CBE and also for subsequent visualization of the graft.

The chicks used were homozygous (B^2B^2) for the major histocompatibility complex. Hence, rejection would not be expected and skin grafts have been completely accepted (7). Histologic examination of the transplant did not reveal a cellular infiltrate characteristic of rejection. Furthermore, some of the lymphocytes which were present were organized into structures resembling bursal follicles. Therefore, it is unlikely that they were part of an inflammatory reaction.

The question of whether the lymphocytes in the graft were of donor or host origin was not directly addressed in this study. Previous work in our laboratory with thymus epithelium has shown that after 16–22 days of culture the tissue is nearly devoid of donor lymphocytes. Those few that remain are dead (10). In this study, histologic and immunofluorescent examination of the bursal epithelium after 13 days in culture revealed essential depletion of lymphocytes. The reappearance of Ig-positive cells after transplantation suggests that they were of host origin.

The results of this study further extend the results reported by Eerola (6). Eerola's finding that atrophy of bursal follicles and the repopulation of cultured fragments following transplantation occur in the reverse order of that seen in ontogenesis was also noted by us. In Eerola's study, however, transplantation onto the chorioallantoic membrane was performed. We employed a wing web site. Establishment of vascular channels and, if necessary, appropriate types of endothelium were necessary for repopulation of our transplants. We have further shown that with time the transplant regains both follicular organization and Ig-positive cells. These morphologic characteristics which were lost in culture may return due to an intrinsic capabililty of the epithelium which is expressed after *in vivo* transplantation or may be the result of secondary contact with lymphocytes. Transplantation of CBE into the wing web of bur-

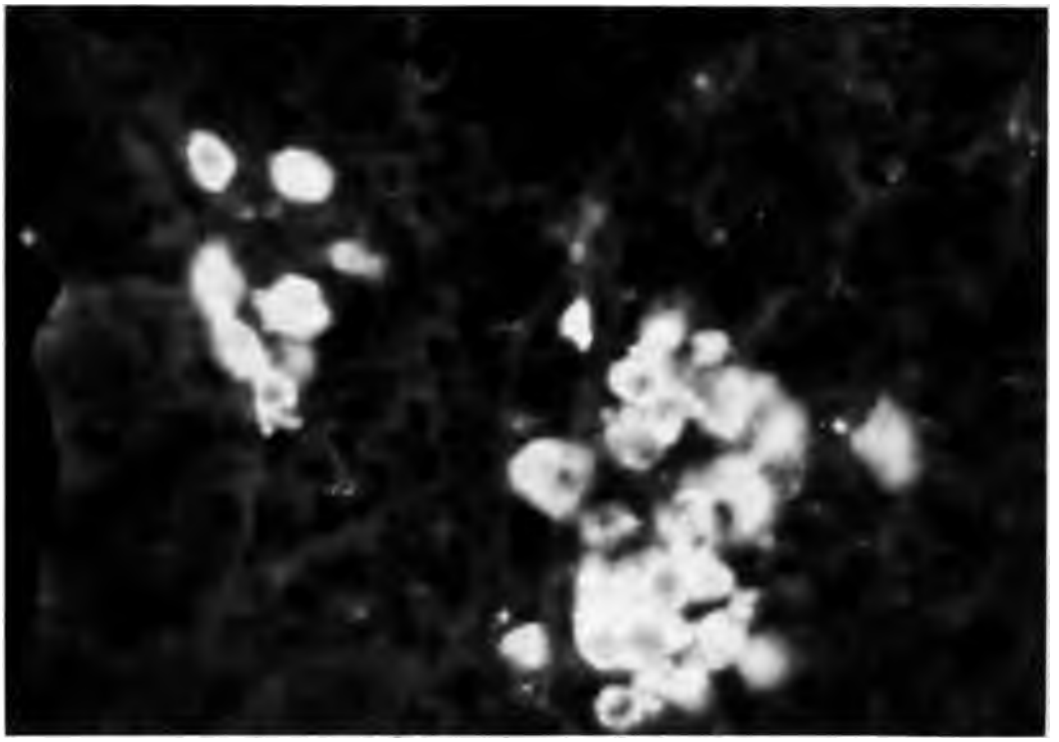


FIG. 8. Immunofluorescence (with anti-μ) of transplant (×308).

sectomized chickens thus may simulate naturally occurring events in B-cell development. Further studies to determine whether the lymphocytes are capable of restoring immune function are in progress.

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1. Cooper MD, Lawton AR, Kincade PW. A two-stage model for development of antibody-producing cells. Clin Exp Immunol 11:143–149, 1972.
2. Ivanyi J. Immunodeficiency in the chicken. I. Disparity in suppression of antibody responses to various antigens following surgical bursectomy. Immunology 28:1007–1013, 1975.
3. Warner NL, Uhr JW, Thorbecke GJ, Ovary Z. Immunoglobulins, antibodies and the bursa of Fabricius: Induction of agammaglobulinemia and the loss of all antibody-forming capacity by hormonal bursectomy. J Immunol 103:1317–1330, 1979.
4. Hong R, Schulte-Wissermann H, Horwitz S. Thymic transplantation for relief of immunodeficiency diseases. Surg Clin North Amer 59:299–312, 1979.
5. Herbert WJ. In: Weir DM, ed. Handbook of Experimental Immunology. Oxford, Blackwell, Vol III:App 4, p26, 1978.
6. Eerola E. *In vitro* culture of chicken bursal epithelium. Cell Immunol 53:162–172, 1980.
7. Stone HA. Use of Highly Inbred Chickens in Research. Tech Bull No. 1514, US Dep Agr, July 1975.
8. Thompson JH, Cooper MD. Functional deficiency of autologous implants of the bursa of Fabricius in chickens. Transplantation 11:71–77, 1971.
9. Bockman DE. Differentiation of epithelium lymphocytes, granulocytes and erythrocytes, in the bursa of Fabricius transplanted to chorioallantoic membrane. Dev Comp Immunol 3:117–125, 1971.
10. Schulte-Wisserman H, Manning D, Hong R. Transplantation of cultured thymic fragments. I. Morphologic and technical considerations. Scand J Immunol 8:387–396, 1978.

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Society for Experimental Biology & Medicine

630 West 168 Street, New York, N.Y. 10032

1983

Constitution and Bylaws

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The Society for Experimental Biology and Medicine

The Society for Experimental Biology and Medicine

CONSTITUTION

ARTICLE I

NAME

The name of the Corporation is The Society for Experimental Biology and Medicine ("the Society").

ARTICLE II

PURPOSES

The Society is formed for the following exclusively charitable, literary and educational purposes:

To promote investigation in the biomedical sciences by encouraging and facilitating interchange of scientific information among disciplines. The principal instruments for achieving this purpose shall be the publication of its *Proceedings*, and where appropriate, the encouragement of sectional and national meetings.

In furtherance of the foregoing purposes, the Society shall have all general powers conferred by law and necessary or incidental to the furtherance of its purposes, subject to the limitation and condition that, not withstanding any other provisions contained herein, the Society shall not have the power to carry on any activities not permitted to be carried on by an organization exempt from federal income taxation under Section 501(c)(3) of the Internal Revenue Code of 1954 (or the corresponding or related provision of any future United States Internal Revenue Law).

The Society shall not carry on propaganda, or otherwise attempt to influence legislation, except to the extent permitted under Section 501 or any succeeding or related Section of the United States Internal Revenue Code. The Society shall not participate or intervene (including the publishing or distributing of statements) in any political campaign on behalf of or in opposition to any candidate for public office.

No part of the net earnings of the Society shall inure to the benefit of any private member or individual, and no officer, director, member, or employee of the Society shall receive or shall be lawfully entitled to receive any pecuniary profit of any kind from the operations of the Society or upon the dissolution of the Society, except reasonable compensation for their expenses or for services rendered the Society in effectuating one or more of its purposes.

In the event of the dissolution of the Society, the balance of all funds, assets, and other property belonging to the Society, after proper payment of all debts, obligations, and liabilities, shall be used and distributed exclusively for one or more of the charitable, literary, and educational purposes set forth in Article II hereof and shall be distributed in accordance with the law to one or more organizations (including, without limitation, organizations of federal, state or local governments) engaged in activities substantially similar to those of the corporation.

The foregoing provisions are intended to comply with Section 501(c)(3) of the Internal Revenue Code of 1954, as amended, and they shall be construed and interpreted in accordance with the meaning of said Section, as the same may be amended from time to time or any comparable or related provision of any succeeding Internal Revenue Law.

BYLAWS

ARTICLE III

OFFICES

Section 1. Principal Office

The principal office of the Society shall be located in the City, County, and State of New York. The Society may establish and maintain offices at any other place or places, within or without the State of New York, as the Council may from time to time determine.

Section 2. Books

There shall be kept at the principal or any other designated office of the Society books or records of membership and correct books of account of the activities and transactions of the Society, including a minute book, which shall contain a copy of the Charter, a copy of these Bylaws and all amendments thereto, and all minutes of meetings of the members and of the Council.

ARTICLE IV
CORPORATE SEAL

The official seal of the Society shall be circular in form and shall contain the name of the Society and the words and figures, "New York—1942".

ARTICLE V
MEMBERS AND MEMBERSHIP

Section 1. Membership

There shall be four classes of membership in the Society: Active, Associate, Student, and Emeritus Members. Except as otherwise provided in these Bylaws, only active members shall be eligible to vote or hold office in the Society.

Section 2a. Active Members

Individuals who have published meritorious original investigations in experimental biology or experimental medicine, and who are actively engaged in experimental research, shall be eligible for active membership in the Society.

New active members may be elected by a vote of the Membership Committee in accord with election rules recommended by the Membership Committee and approved by the Council from among nominations submitted by the Executive Secretary pursuant to Article VII, Section 7, hereof, at any meeting of the Committee. Members of the Board of Editors shall be active members of the Society by virtue of their position as members of the Board of Editors.

Section 2b. Associate Members

Eligibility: Individuals who are engaged in research in experimental biology or medicine and/or teaching or administration in these areas. They must be sponsored by two regular members of the Society and must be residents of North America.

Length of membership: No restriction. They may later be proposed for regular membership.

Dues: Same as regular members. Dues include a subscription to the *Proceedings*.

Duties and privileges: Same as for regular members, *except* for the privileges of

1. Holding offices, or membership on certain committees.
2. Voting.
3. Sponsoring new members.
4. Sponsoring manuscripts or abstracts.

Section 2c. Student Members

Eligibility: Any student who is a candidate for a doctorate degree and is actively engaged in research in experimental biology or medicine, as attested to by two regular members of the Society (only one of whom needs to write a letter, but both need to sign the application), and who is a resident of North America.

Length of membership: No individual may remain in this category for more than three years unless an extension is granted on the request of his/her sponsor.

Dues: $5.00. Dues include a subscription to the *Proceedings*.

Duties and privileges:

1. Register to attend SEBM Scientific Meetings at student rates.
2. Student membership in the Regional Section, where applicable.

Section 3. Emeritus Members

Any active member in good standing, who retires at the age limit of his institution or who is retired because of illness, may upon request be declared an emeritus member by the Council and may be relieved of payment of the annual dues. An

emeritus member may submit manuscripts and sponsor the submission of manuscripts by non-members for publication in the *Proceedings*.

Section 4. Annual Meeting of Active Members

The annual meeting of the active members shall be held at such time and place as the Council may from time to time determine and as shall be designated in the notice of such meeting.

Section 5. Notice of Meetings

Written notice of all meetings of active members, stating the time, place, and objects thereof, shall be served personally or by mail at least ten (10) and not more than forty (40) days before such meeting to each active member entitled to vote thereat at his or her address as the same appears on the books of the Society.

Section 6. Quorum

At all meetings of active members of the Society, 100 active members, present in person or represented by proxy, shall constitute a quorum. If, however, such a quorum shall not be present or represented at any meeting of the active members, the active members entitled to vote thereat, present in person or by proxy, shall have power to adjourn the meeting to another time, without notice other than announcement at the meeting, whereupon at any such meeting, provided a quorum is present, any business may be transacted which might have been transacted at the meeting as originally called. A majority vote of the active members in attendance or represented by proxy, provided a quorum is present, shall constitute a valid vote.

Section 7. Action Without Meeting

Action of the active members may be taken any time, without a meeting, by the unanimous written consent of all the members to a resolution authorizing such action and any such action may be so taken which might have been taken at a meeting duly called. Any such action or resolution shall be recorded by the Executive Secretary in the book containing the records of meetings of the active members and shall have the same force and effect as if adopted at a meeting of the active members duly called and held.

Section 8. Regional Groups of Members

Members of the Society residing in the same regions may organize under their own rules, subject to the approval of the Council, to sponsor scientific meetings. The Society will provide such groups, upon request, with the names and addresses of members residing in their region and provide such other assistance as the Council may approve.

ARTICLE VI
THE COUNCIL

Section 1. Number and Term of Office

The Council shall consist of the President, the immediate Past President, the President-Elect, the Treasurer, the Chairman of the Publication Committee, the Chairman of the Membership Committee, the Executive Secretary (*ex officio*), and twelve additional councillors, three of whom shall be elected annually by the active membership from the slate submitted by the Executive Secretary pursuant to Article VII, Section 7, hereof, for a term of four years commencing on July 1, next following their election and until their successors shall have been duly elected and shall have qualified. In the event of a tie-vote, election shall be resolved by majority vote of the Council.

Section 2. Duties and Powers

The entire direction and management of the affairs of the Society shall be vested in its Council which shall have complete discretion to determine the relation and

obligation of members to the Society and to each other and all expenditures, disbursements, or distributions to be made in carrying out the purposes of the Society. The Council shall approve the slate of nominees for councillors and officers to be submitted to the active membership from among those included on the slate prepared by the Nominating Committee pursuant to Article VIII, Section 3 thereof. The Council shall include on such slate any nomination for any office submitted by 25 active members of the Society.

Section 3. Removal of Councillors

Any Councillor may be removed due to conduct injurious to the Society or contrary to the best interests of the Society by: (i) two-thirds vote of the active members voting; or (ii) by a majority vote of the non-accused Councillors at the meeting of the Councillors at which such action is taken. The accused member shall be given an opportunity for a hearing before the Council.

Section 4. Vacancies

In case of any vacancy in the Council, the remaining Councillors may at any meeting of the Council or by unanimous written consent elect a successor to hold office for the unexpired term of the councillor whose place shall be vacant and until the election and qualification of his successor.

Section 5. Annual Meetings

The Council shall hold a regular annual meeting at a time in close proximity to the annual meeting of the active members.

Section 6. Special Meetings

Special meetings of the Council shall be called by the Executive Secretary whenever directed by the President or by any four members of the Council.

Section 7. Notice

Notice of the place, day, and hour of any regular meeting of the Council and notice of the place, day, and hour and purposes of every special meeting of the Council shall be given to each Councillor at least ten (10) days previous to such meeting, by delivering the same to him or her personally or by telegraphing or by mailing such notice addressed to his or her address as the same appears on the books of the Society. It shall not be requisite to the validity of any meeting of the Council that notice thereof shall have been given to any Councillor who is present thereat or who, if absent, waives notice thereof in writing, either before or after the holding thereof.

Section 8. Place of Meeting

The Council may hold its meetings at such place or places within or without the State of New York as the Council or the President may from time to time determine.

Section 9. Quorum

A majority of the Councillors then holding office shall constitute a quorum for the transaction of business, but less than a quorum may adjourn any meeting until a quorum is present. No notice of any adjourned meeting of the Council need be given.

Section 10. Manner of Acting

Except as otherwise provided by statute, by the Charter, or by these Bylaws, the action of a majority of Councillors present at any meeting at which a quorum is present shall be the act of the Council.

Section 11. Action Without Meeting

Action of the Council or any committee thereof may be taken any time without a meeting by the unanimous written consent of all the Councillors or members of the committees to a resolution authorizing such action, and any action may be so

taken which might have been taken at a meeting duly called. Any such action or resolution in writing signed by all the Councillors or members of the committees and records of meetings of the Council or committees thereof shall have the same force and effect as if duly adopted at a meeting of the Council or committee duly called and held.

Section 12. Annual Report

The Council, pursuant to Section 519 of the Not-For-Profit Corporation Law, shall present at the annual meeting of the active members and file with the minutes thereof a report, duly verified or certified and containing the information as provided by Section 519, or any successor section.

ARTICLE VII
OFFICERS

Section 1. Enumeration

The officers of the Society shall be a President, a President-Elect, a Treasurer, an Executive Secretary, and such additional officers as the Council may from time to time elect.

Section 2. Election and Term of Office

The officers other than the President and the Executive Secretary shall be elected biennially pursuant to Article VII, Section 7, at the annual meeting of the active members from among names submitted by the Nominating Committee pursuant to Article VIII, Section 3 hereof and shall hold office for two years commencing on July 1 next following their election and until their successors are duly elected and have qualified unless removed prior to such time. In the event of a tie-vote, election shall be resolved by vote of the Council. The President-Elect shall succeed to the office of President upon expiration of his term as President-Elect.

Section 3. Removal of Officers

The Council shall have the right to remove any officer for conduct injurious to the Society or contrary to the best interests of the Society at any time by a majority vote of the non-accused members of the Council. The accused officer shall be given an opportunity for a hearing before the Council.

Section 4. President

The President, except where otherwise directed by the Council, shall be the chief executive officer of the Society. He shall preside at all meetings of members and of the Council and shall be an ex-officio member of the Publication Committee. He shall perform all duties incidental to his office and such other duties as may be delegated by the Council.

Section 5. President-Elect

During the absence, incapacity, or disability of the President, the President-Elect shall exercise all the functions of the President and, when so acting, shall have all the powers of and be subject to all the duties of and restrictions upon the President. In the event that the office of the President becomes vacant, the President-Elect shall succeed to that office for the remainder of its term and shall not be barred from serving as President for the next succeeding term. A new President-Elect shall be elected at the next annual meeting of active members following the assumption by the President-Elect hereunder of the office of President. The President-Elect shall be an *ex officio* member of the Publication Committee and shall have such other powers and discharge such duties as may be assigned to him from time to time by the Council.

Section 6. Treasurer

The Treasurer shall be responsible for the general supervision of the financial matters of the Society. He shall render to the President and Councillors whenever they may require an account of all transactions and of the financial condition of

the Society and shall submit an annual report to the Council for distribution to the active members.

Section 7. Editor/Executive Secretary

The Editor shall be appointed by the President in consultation with the Publication Committee and with the approval of the Council. The Editor shall be responsible to and serve at the pleasure of the Council. The Editor and the Board of Editors shall be responsible for the editorial conduct of the *Proceedings*. He or she may serve as Executive Secretary of the Society with the approval of the Council and shall be an *ex officio* member of the Council and of all standing committees of the Society and shall preside at all meetings of the Society and the Council in the absence of the President and President-Elect. The Editor shall be compensated at such salary as may be determined by the Council.

The Executive Secretary shall submit to each active member at least 90 days in advance of the annual meeting of active members the slate of nominees approved by the Council under Article VI, Section 2 hereof. Within 60 days of the mailing of such slate any group of 25 active members may submit additional nominations to the Executive Secretary. Thirty days in advance of the annual meeting of the active members the Executive Secretary shall submit to each active member a ballot including the slate approved by the Council and any nominations proposed by active members hereunder. No distinction shall be made on such slate between nominees proposed by the Nominating Committee and approved by the Council and those proposed by active members. The active members of the Society shall vote on the nominations in person at the annual meeting or by proxy. Proxy votes must be returned to the Society office by one week before the annual meeting in order to be valid. The Executive Secretary shall ask two active members of the Society to serve as tellers. The votes shall be tallied and certified by the tellers, at the annual meeting. A plurality of votes shall be sufficient for election.

The Executive Secretary shall submit to the Membership Committee applications for membership in the Society of individuals proposed by any two or more active members of the Society and shall forward such applications to the Committee together with documentation of the qualifications of such applicants.

The Executive Secretary shall submit an annual report to the Council for distribution to the active members, attend to such correspondence as may be assigned to him or her, perform all the other duties incidental to the office of the Executive Secretary and shall keep the minutes of the meetings of members. The Executive Secretary shall attend to the giving and serving of all notices on behalf of the Society as the Councillors may direct. The Executive Secretary shall also keep a record of all members of the Society, alphabetically arranged, showing their place of residence and the time when they became members and perform all other duties incidental to the office of the Executive Secretary.

The Executive Secretary shall have the care and custody of all the funds and securities of the Society and shall deposit the same in the name of the Society in such bank or trust company as the Council may select. He shall sign all checks, drafts, notes, and orders for the payment of money and shall pay out and dispose of the same when, as, and if authorized to do so by the Council and shall keep full and accurate accounts of receipts and disbursements in the books of the Society. The Executive Secretary shall be responsible for the collection of dues and receipt of such Society funds, and for the disbursement of funds as determined by the Treasurer and Finance Committee. He shall give such bond for the faithful performance of his duties as the Society may determine.

Section 8. Vacancies

In the case of vacancy in any office, the Councillors may at any meeting of the Council or by unanimous written consent elect a successor to hold office for the unexpired term of the officer whose place shall be vacant and until the election and qualification of his successor.

ARTICLE VIII
COMMITTEES

Section 1. Authority

The Council may by general resolution delegate to committees of its own number or to officers of the Society such powers as it may see fit. All committees shall be responsible to the Council for their actions and decisions.

Section 2. Standing Committees

There shall be the following standing committees: Nominating, Publication, Board of Editors, Finance, and Membership.

Section 3. The Nominating Committee

The Nominating Committee shall consist of at least three and no more than seven active members who shall be appointed by the President with the approval of the Council for a term of two years and until the appointment and qualification of their successors. Due consideration shall be given to regional representation on this Committee.

The Committee shall, biennially, present to the Council at its meeting next preceding the annual meeting of the active members, a slate of nominees consisting of at least two active members for President-Elect and Treasurer, each to serve for two years and, annually, a slate of six or more active members for Councillors with three to be elected to serve for four years. A person who has served on the Council may be included in the slate of nominees for Councillor only if two years have elapsed since the completion of his previous term.

Section 4. Publication Committee

The Publication Committee shall consist of four members and a chairman who shall be active members of the Society and shall be appointed by the President with the approval of the Council for a term of five years and until the appointment and qualification of their successors. No member may be appointed to serve a second term until two years following the completion of his first term, and no member may serve more than two terms.

The Publication Committee shall be responsible for consulting with the Editor and the Council regarding major editorial policy decisions of the Society and shall report to the Council at its annual meeting on editorial policy and publication matters.

The members of the Publication Committee shall confer with and advise the Editor on matters pertaining to the publication of the *Proceedings*. The Committee shall meet periodically with the Editor and at such other times as the Chairman of the Committee or President of the Society shall designate. The Committee may transact by mail such business as can not be transacted conveniently at meetings.

The Committee shall solicit names of individuals for appointment to the Board of Editors from current and retiring editors in pertinent scientific fields and from the Editor and shall submit a list of nominees to the Council for approval. The Councillors may also nominate editors. Nominees need not be members of the Society.

Section 5. Board of Editors

The Board of Editors shall consist of not less than twenty members appointed by the Council from among the nominees submitted by the Publication Committee for a term of three years and until the appointment and qualification of their successors.

The Board of Editors shall review papers for publication in the *Proceedings*.

Section 6. Finance Committee

The Finance Committee shall consist of the Treasurer as Chairman and four additional members who need not be active members of the Society, two of whom shall be appointed by the President and approved biennially by the Council at its

meeting preceding their end of term for a term of four years and until the qualification of their successors.

The Finance Committee shall supervise the finances of the Society, shall review the annual budget prepared by the Executive Secretary and shall submit such budget to the Council. The Committee shall hold a minimum of one meeting each year for review of the budget. The Committee shall be responsible for the appropriate disbursement of funds by the Treasurer and Executive Secretary as dictated by the budget and for the investment and management of all assets of the Society.

Section 7. Membership Committee

The Membership Committee shall consist of eight active members, four of whom shall be appointed biennially by the President and approved by the Council at the meeting of the Council next preceding the annual meeting of the Society for a term of four years and until the appointment and qualification of their successors. The Chairman of the Membership Committee shall be appointed by the President and approved by the Council. The Committee shall review applications for membership in the Society submitted by the Executive Secretary and shall elect new members to the Society in accordance with Article V, Section 2 and pursurant to Article VII, Section 7, hereof.

The Membership Committee shall meet annually to consider changes in acceptance procedures for recommendation to the Council and to reconsider special deferred cases for nomination for membership. The Committee may transact by mail such business as can not be transacted conveniently at meetings.

Section 8. Special Committees

The President, with the approval of the Council, may from time to time appoint such special committees as he deems appropriate and necessary to assist in the administration of the Society's activities.

ARTICLE IX
CONTRACTS, CHECKS, NOTES, BANK ACCOUNTS, ETC.

All contracts of the Society and all checks and drafts and other orders for the payment of money out of the funds of the Society and all promissory notes and other evidence of indebtedness of the Society shall be signed on behalf of the Society by such officer or officers, agent or agents, and such manner, as shall from time to time be determined by resolution of the Council.

ARTICLE X
DUES AND ASSESSMENTS

Section 1. Fixing of Dues

The Council shall fix dues and assessments which shall be paid by members other than members of the Board of Editors of the Society.

Section 2. Arrears

An active member who is in arrears for dues for two consecutive years or for publication costs for one year shall, following thirty days written notice by the Executive Secretary, cease to be an active member unless excused by the Council or reinstated by the Council upon payment of such arrears.

ARTICLE XI
PUBLICATIONS

Section 1. The Proceedings

The official organ of the Society shall be the *Proceedings of the Society for Experimental Biology and Medicine*.

Section 2. Charges

Authors shall be charged for space in the *Proceedings* at rates to be determined by the Council.

Section 3. Receipt of the Proceedings

All active, associate, and student members shall receive the *Proceedings*. Emeritus members may subscribe to the *Proceedings* at the rate paid by active members.

ARTICLE XII
PARLIAMENTARY AUTHORITY

All meetings of the Society, the Council, Standing Committees, and special committees thereof shall be governed by the rules contained in "Roberts Rules of Order, Revised" in all cases to which they are applicable except when such rules are inconsistent with the Bylaws of the Society.

ARTICLE XIII
AMENDMENTS

Amendments to these Bylaws may be proposed by the Council on its own initiative or upon the application of any 50 active members of the Society. Notice of any proposed amendment shall be sent by the Executive Secretary to each active member of the Society at least 60 days in advance of the annual meeting of active members. These Bylaws may be amended by a majority vote of the active members voting in person or by proxy at such meeting. Proxies must arrive at the Society office prior to one week before the meeting to be considered valid. Approved amendments shall be effective as of the following July first.

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    2. Abramson DI. Circulation in the Extremities. New York, Academic Press, p000, 1967.
    3. Newell A. Simon HA. Programs as theories of higher mental processes. In: Stacy RW, Waxman BD, eds. Computers in Biochemical Research. New York, Academic Press, Vol 2:p000, 1965.

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centimeter	cm	milliosmole	mOsm
counts per minute	cpm	minute	min
cubic centimeter	cm^3	molal (concentration)	*m*
Curie	Ci	molar (concentration)	M
degree Celsius (Centigrade)	°C	mole	spell out
degree Fahrenheit	°F	molecular weight	mol wt

diameter	diam	nanogram	ng
gram	g	nanometer	nm
hour	hr	normal (concentration)	*N*
inch	in	osmole	Osm
inside diameter	i.d.	ounce	oz
intramuscular	im	outside diameter	o.d.
intraperitoneal	ip	parts per million	ppm
intravenous	iv	percent	%
kilocalorie	kcal	picogram	pg
kilogram	kg	revolutions per minute	rpm
liter	spell out	second	sec
meter	m	specific activity	sp act
microliter	μl	square centimeter	cm^2
micrometer	μm	square meter	m^2
milligram	mg	subcutaneous	sc
milliliter	ml	volt	V
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